Starchy foods in the prevention of type 2 diabetes mellitus

Effects of food structure and composition on starch digestibility and the postprandial metabolic response

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General introduction

1.1 THE IMPORTANCE OF LIFESTYLE-RELATED FACTORS IN THE PATHOGENESIS OF T2DM

Type 2 Diabetes Mellitus (T2DM), also known as non-insulin dependent diabetes mellitus, is a rapidly growing health problem affecting many people worldwide. It is estimated that around 450 million adults (18-99 y) currently have diabetes, of which around 90% concerns T2DM (1). This worldwide prevalence is rapidly increasing, and was estimated to reach around 693 million people in 2045 (1). The development of T2DM is often preceded by overweight or obesity and insulin resistance (IR), and is implicated with an increased risk of cardiovascular diseases (CVD) (2). The metabolic disease mainly develops in elderly persons, but it is increasingly affecting young persons as well (3). This worrying trend shows the urge for preventive measures.

Dysregulation of glucose homeostasis

In T2DM glucose homeostasis is affected, which leads to serious health consequences. High glucose concentrations in the blood (hyperglycemia) can cause damage to the heart, blood vessels, kidneys, eyes and nerves (4). Too low concentrations of glucose (hypoglycemia) can lead to fainting and even coma.

In healthy individuals, glucose concentrations in the blood are kept closely within a certain range by regulatory hormones to prevent health problems. The liver has a prominent role in glucose homeostasis, being able to store excess glucose in the form of glycogen and release it again in the period between meals when glucose levels decrease. Because glucose is an important fuel for the human body, these processes are tightly regulated, mainly by the pancreatic hormones insulin and glucagon. In the normal postprandial state when glucose enters the body via nutrition, insulin is excreted from the β-cells of the pancreas in response to the increasing glucose concentrations (5). This increases glucose uptake by insulin-sensitive tissues, such as muscle and adipose tissue, resulting in normalization of glucose levels in the blood. Glucose is partly oxidized in the cells to provide energy and a part can be stored in muscle or liver cells in the form of glycogen. Excess amounts of glucose can be stored as fat due to lipogenesis (6).

Over time, influenced by factors such as age, gender, genetics, lifestyle and body weight, tissue cells can become less sensitive to insulin (7). This insulin resistance (IR) results in increasingly high glucose levels in the blood. Because the body attempts to keep the postprandial glucose concentrations within range, the β-cells need to produce and secrete more insulin. This process can continue unnoticed, but the high glucose and insulin concentrations are detrimental for the body. Compared to other tissues, β-cells are even more vulnerable when it comes to glucose toxicity, because glucose can enter these cells independent of insulin (equilibrium via transporter GLUT2) (8). Eventually the pancreas can no longer make sufficient amounts of insulin due to β-cell deterioration/failure (9, 10). In this stage, where diabetes has developed, patients are advised to take glucose lowering medication and/or insulin to manage their disease.

Thus, T2DM is characterized by hyperglycemia, insulin resistance, and relative impairment in

insulin secretion. The progression from normal-to-abnormal glucose homeostasis is a continuous one, with the term pre-diabetes describing the intermediate stage (11). Pre-diabetes is a condition in which blood glucose levels are higher than normal, but not high enough to be diagnosed as type 2 diabetes. The stage of pre-diabetes is characterized by either high fasting glucose concentrations related to hepatic IR, named impaired fasting glucose, and/or by postprandial hyperglycemia due to peripheral IR and/or inadequate insulin production, indicated as impaired glucose tolerance (IGT) (12). In addition to the high prevalence of T2DM, the International Diabetes Federation estimated in 2015 there are 318 million adults with impaired glucose tolerance, which puts them at high risk of developing T2DM in the future (13).

Role of lifestyle-related factors in the pathogenesis of T2DM

Although genetic elements are involved in the pathogenesis of T2DM, there is an important role for lifestyle-related factors. In the current western society the onset of T2DM is often related to a sedentary lifestyle and overweight; around 80% of persons with diabetes is overweight or obese (14). This indicates that a lack of physical activity and a poor diet are important modifiable risk-factors of the disease. Therefore, next to pharmaceutical intervention, persons with diabetes are generally advised to improve their lifestyle by increasing physical activity in combination with a healthy diet, with moderate weight loss as an important goal.

Persons with pre-diabetes (especially IGT) may be able to delay or even prevent the progression of the disease by adapting their lifestyle, because the progression from pre-diabetes to T2DM is rather slow, and the process is reversible until a certain stage (15). A study on the impact of lifestyle intervention on glucose metabolism, with three years of follow-up (SLIM-study, Maastricht) was conducted in 147 subjects with IGT (16). Lifestyle changes consisted of both a dietary element and a physical activity element based on general public health recommendations. Dietary recommendations were based on the Dutch Guidelines for a healthy diet (Dutch Nutrition Council) and participants were instructed to increase physical activity to at least 30 min 5 times a week. The intervention resulted in improved body weight, improved measures of steady-state insulin sensitivity (IS) and a beneficial effect on 2h glucose and free fatty acid concentrations after an oral glucose tolerance test (OGTT). Diabetes incidence was reduced by 58% in the intervention group (16). Similar effects were obtained in other European studies, and pooled analysis clearly showed the increasing preventive effects of ≥5% weight loss maintained for 1 up to 3 years (17).

Nutrition is an important focus area for prevention of the development of T2DM, mainly for persons at risk, but for healthy individuals as well. In observational studies several characteristics of a diet were associated with an altered risk of the development of T2DM. A recent systematic review formulated evidence based dietary recommendations for both the prevention and management of T2DM (18), as the aims for nutritional strategies are similar; to achieve optimal blood glucose and blood lipid concentrations, to prevent, delay, and/or treat diabetes-related complications, and to improve health through balanced nutrition. Healthy dietary patterns were typically rich in whole

grains, fruits and vegetables, nuts and legumes; moderate in alcohol consumption; and lower in refined grains, red or processed meats, and sugar-sweetened beverages. Although there is some debate about the relative amount of carbohydrate, fat and protein intake which is optimal in the prevention and management of T2DM, it was emphasized that the quality of consumed fats and carbohydrates is more important than the relative quantity of these macronutrients (18, 19).

Quality of carbohydrates and (prevention of) T2DM

Dietary intake of carbohydrates accounts for large fluctuations in circulating glucose concentrations. Carbohydrates differ substantially in their physiological and metabolic response depending on their source and accompanying food matrix (e.g. sugar-sweetened beverages vs whole grain bread), and therefore have varying health effects. The quality of carbohydrates and carbohydrate-rich foods in the diet is therefore very important (19). The rise in blood glucose concentration following the consumption of a carbohydrate-rich food product, often reflected using the Glycemic Index (GI) (20), is seen as one of the indicators of carbohydrate quality (21). The GI is determined using the incremental area under the curve (iAUC) of the 2h glucose response, compared to the iAUC of a reference food (white wheat bread) or an equivalent amount of glucose (**Figure 1**). With this classification system based on the products' glycemic impact, a high GI product is indicated by a value above 70, a low GI product has a value below 55 (20). Low GI products often contain slowly digestible carbohydrates, and therefore the terms low/high GI and slowly/rapidly digestible carbohydrates are often used interchangeably.

The GI was intended as a tool for T2DM patients to avoid postprandial hyperglycemia. Later, the concept of glycemic load (GL) was developed to simultaneously describe the quantity of carbohydrate in a food serving, meal, or diet. Foods with a low GI and/or GL can contribute to improved glycemic control (22), making them suitable for use in the management of T2DM.

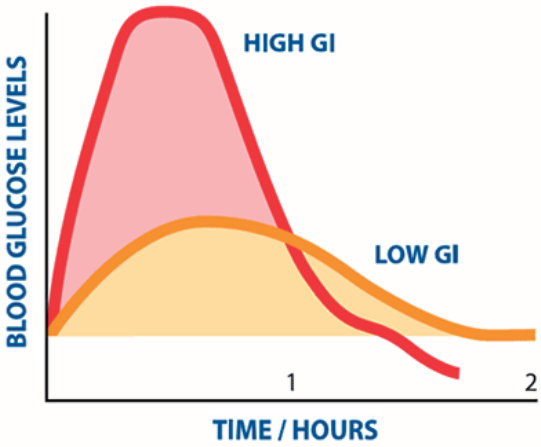

Figure 1. *Illustration of a high and a low glycemic response over time. Adapted from The University of Sydney.*

However, products with a low glycemic response might also be beneficial for healthy individuals or persons at increased risk of developing T2DM when it comes to prevention of the disease. Several epidemiological studies have found low GI and/or low GL diets to be associated with a reduced risk of developing obesity, IR, T2DM and CVD (23-30). As an example related to T2DM, large studies by Salmeron et al. showed that GI and GL were positively associated with the risk of diabetes, whereas cereal fiber intake was inversely associated with risk of diabetes in both men (23) and woman (24).

The combination of a high glycemic load and a low cereal fiber intake further increased the risk of diabetes (RR=2.50, 95% CI, 1.14-5.51) when compared with a low glycemic load and high cereal fiber intake. The findings suggest that consuming grains in a minimally refined form can reduce the incidence of diabetes (23, 24). A meta-analysis of observational studies concluded that low-GI and/or low-GL diets were independently associated with a reduced risk of diabetes and heart disease, with the protection being comparable with that seen for whole grain and high fiber intakes (31). However, there are some inconsistencies in the literature, as some other epidemiological studies found no association between GI and the risk for T2DM (32, 33).

Several (short term) intervention studies with low and high glycemic foods or diets were performed to study the effect on body weight and metabolic health, evaluating intermediate risk markers for T2DM, as reviewed by Blaak et al. (34). Overall, these studies and their results are very heterogeneous. It was noted that several studies for instance did not report on the actual glycemic response of their intervention diet or did not find a difference in glycemic response between their low and high-GI diet. This makes it difficult to compare and draw conclusions on the effects of low- and high GI diets. However, a systematic review and meta-analysis (including 45 studies) by Livesey et al. evaluating the impact of the glycemic response on markers of health, reported that diets with a low-GI reduced fasting blood glucose and glycated protein levels (35). Several included studies found increased IS, using a variety of measurement methods, in non-diabetic subjects on a low-GI diet.

To get more information on this possible association between postprandial glucose concentrations and the risk of developing IR and T2DM, data from long term intervention studies that aimed to lower postprandial glycemia by pharmaceutical intervention in persons with IGT could be used. For instance, acarbose is an α-glucosidase inhibitor that decreases the breakdown of complex carbohydrates such as starch and thereby delays glucose absorption and reduces postprandial glycemia. An example of an intervention study with acarbose is the STOP-NIDDM trial, in which over 1400 persons with IGT received either 100 mg acarbose or placebo 3x daily (36). They found that with acarbose the risk of progression to T2DM over 3.3 years was decreased by 25%. In addition, an increased probability of reversing IGT to NGT over time was observed. These results indicate that reducing starch digestibility and alleviating postprandial hyperglycaemia delays or prevents the progression to T2DM. A meta-analyses of similar studies (n=5) reported that due to acarbose treatment on average a 20% reduction in incidence of T2DM in patients with IGT was found (37).

From these studies it is difficult to determine whether it is the lower glycemic response itself or other associated responses, such as a lower insulin response, that causes the beneficial health effects. It is however clear that these high postprandial responses, even in healthy subjects, result in a deleterious metabolic and hormonal state, harming several organ systems, with the phenomena being exacerbated in subjects with IGT (34). Thus, there are indications that repeated high postprandial glucose concentrations (and/or related factors) may be implicated with an increased risk of the development of obesity, IR, T2DM, and CVD. Hence, the reduction of postprandial glucose excursions is considered beneficial for health. This could be targeted by nutritional strategies, with a focus on food products rich in starch.

1.2 STARCHY FOOD PRODUCTS AND THEIR POSTPRANDIAL RESPONSE

Many plants and crops such as wheat, corn, rice and potatoes are rich in starch, which is the main form by which plants store carbohydrates, and therefore are widely used as staple foods worldwide. Consumption of foods rich in starch have a direct impact on glucose homeostasis. These foods, depending on their characteristics, might be related to the prevention or development of IR and T2DM.

This section will provide information about starch digestibility and uptake of starch-derived glucose, and about possible ways to influence these processes in order to decrease the postprandial glycemic response. To be able to monitor the effects of modifications of starch digestibility dedicated techniques are necessary, which will be subsequently discussed.

Glycemic response of wheat products

One of the most common sources of starch in human nutrition is wheat (*Triticum* spp.). Several types of products are made from wheat, which can be either high, intermediate or low glycemic (38). In general, consumption of a wheat-based product such as pasta results in a low, prolonged elevation of postprandial glucose concentrations (low GI). However, the consumption of wheat bread often results in high postprandial glucose concentrations (high GI) and the starch in wheat bread is therefore considered to be rapidly digested. This mainly accounts for refined white wheat bread, which is often used as a high glycemic reference food. However, although delivering more beneficial nutrients and fibers, the glycemic response of whole-grain wheat bread is generally not much lower (38). Therefore, there is some room for improvement when it comes to the glycemic response to wheat bread. When attempting to decrease the glycemic response to a food product, the most obvious factor to approach is the digestibility of starch in the product, which mainly depends on the intrinsic characteristics of the starch and on factors related to the food product.

Digestion of starch and uptake of starch-derived glucose

Starch is a polysaccharide consisting of many glucose units joined by glycosidic bonds. The two glucose polymers that build up starch are amylose and amylopectin. Amylose has a linear structure

due to α-1,4 bonds between the glucose units, whereas additional α-1,6 bonds in amylopectine result in a branched structure (39, 40). When starchy food products are consumed, starch is broken down by enzymes that cleave the bonds between the sugar units. During mastication, the first enzymatic step is α-amylase in the saliva which starts the breakdown of α-1,4 bonds in starch. After further destructuring of the food and bolus formation in the stomach, this breakdown is continued in the small intestine, where the pancreas excretes several digestive enzymes, including α-amylase. Enzymes present in the brush border of the small intestine (e.g. (iso)maltase, α-glucosidases), continue with this breakdown by splicing e.g. the di-saccharide maltose into two glucose units. This is the final step of digestion, before the resulting glucose molecules can be taken up by the intestinal cells. Glucose enters the enterocytes via the sodium-dependent hexose transporter SGLT-1 via active transport. Glucose leaves the enterocytes via the basolateral membrane using the GLUT2 transporter for facilitated diffusion to enter the portal venous system.

Factors that affect the rate of starch digestion and intestinal uptake of starch-derived glucose

Physiological processes

The rate and extent of starch digestion and intestinal uptake of starch-derived glucose can be affected by several physiological processes. The first step in digestion, oral processing, is highly dependent on the chewing habits (e.g. duration, thoroughness) of a person, the amount of saliva production and also on the structure of the food product (41). Gastric emptying of the resulting food bolus is also a modifiable factor, which is determined by e.g. volume, caloric content, the particle size, the fat content and the viscosity of the food bolus (42). Further, gastric emptying is regulated by several gastro-intestinal hormones. Once the food bolus enters the small intestine, there can be an additional difference in intestinal transit time. This is influenced by factors including gastro-intestinal hormones, viscosity of the food bolus, intake of a subsequent meal and person-related factors (43, 44). The resulting contact time between the nutrients and the intestinal mucosa can affect the rate and extent of starch digestion and/or the uptake of starch-derived glucose (45, 46).

Thus, several physiological processes, whether or not influenced by the food product, can influence starch digestibility and the uptake starch-derived glucose. In addition, many product-related factors determine the rate of starch digestion and the uptake of starch-derived glucose.

Product-related factors

Starchy food products differ in the rate of starch digestion and/or uptake of starch-derived glucose. This can depend on several product-related factors, which can be modified in order to influence the postprandial response. As indicated, starch consists of both amylose and amylopectin, and the proportion depends on the botanical origin of the starch. Amylose, generally making up around 30% of starch, is more resistant to digestion due to its linear structure (39). A possibility to decrease starch digestibility is therefore by increasing the amylose/amylopectin ratio.

Other starch characteristics additionally depending on the botanical source, determine the

rate of digestion as well. In plants, amylose and amylopectin are organized in concentric layers of alternate crystalline and amorphous regions, which form a starch granule. The structural characteristics of starch granules, such as granule size and the unit-chain length distribution of amylopectin, modulate their enzyme digestibility (47, 48).

When raw starch is prepared for consumption by heating in the presence of water, e.g. cooked, or baked, starch granules will become gelatinized. This process involves disruption of the semi-crystalline granular structure and leakage of starch molecules from the granules, which increases the accessibility of starch for hydrolysis (or amylolysis) (39). The degree of gelatinization depends on water content, temperature, duration of heating and the pressure (49). Cooling down and ageing of gelatinized starch (e.g. pasta, or bread, respectively) results in re-crystalinization. This rearrangement process is called retrogradation and results in a decreased starch digestibility and an increase in resistant starch (50). The formation of resistant starch has an additional health benefit, as it is suitable for fermentation by the colonic microbiota resulting in short-chain fatty acids (51). The presence of organic acids in bread (e.g. sourdough) may increase starch retrogradation and thus resistant starch content (52).

After processing of the food product, the physical form of the food may hinder the accessibility of starch as well. The mastication process, which is partly determined by the food structure, might influence this. For instance, a compact food structure and the larger particle size remaining after chewing and gastric processing, both limit the surface area for digestive enzymes to gain access to the substrate, thereby limiting the rate of starch digestion (53). The importance of food structure was demonstrated in a study where bread and pasta were both made with pasta ingredients (54). The compact structure of pasta achieved during processing contributed substantially to a decreased starch hydrolysis and glucose response, as indicated by the finding that bread made from durum wheat gave a higher glucose response compared to pasta made from the same ingredients. Here, the food matrix is another factor that may explain the slow digestion of starch in pasta. The presence of a continuous protein matrix or network, which entraps starch granules, could limit and/or retard the accessibility of starch to α-amylase (55).

Kernels or larger grain particles in the food product could also keep the starch protected from the digestive enzymes (56-58). Several studies showed that substitution of flour with boiled cereal grains or parboiled cracked wheat in bread could reduce the postprandial glycemic response compared to white wheat bread (56, 57, 59, 60). In addition, the presence of other components in the food product or the meal can affect starch digestibility. For example, the presence of phytochemicals that could inhibit the digestive enzymes (α-amylase, α-glucosidase) (61), such as polyphenol-rich ingredients (62) or extracts from white beans (63). The fat content of the product or co-ingestion of fat with the meal may decrease gastric emptying, and thereby delay the digestion process, generally resulting in a lower glycemic response (64). The same may account for the addition of acid to a meal or ingesting sourdough bread (65). The fiber content of the food product and the type of fiber can also influence starch digestibility and glucose uptake. Soluble or viscous fiber is able to

increase viscosity of the food bolus, and may thereby interfere with starch digestion by decreasing contact between starch and digestive enzymes. In addition, the rate of absorption of glucose can also be delayed by the presence of viscous fiber by hindering glucose transport to the brush border membrane and by thickening the unstirred layer (43).

The main factors involved in the digestion of starch are summarized in **Figure 2**.

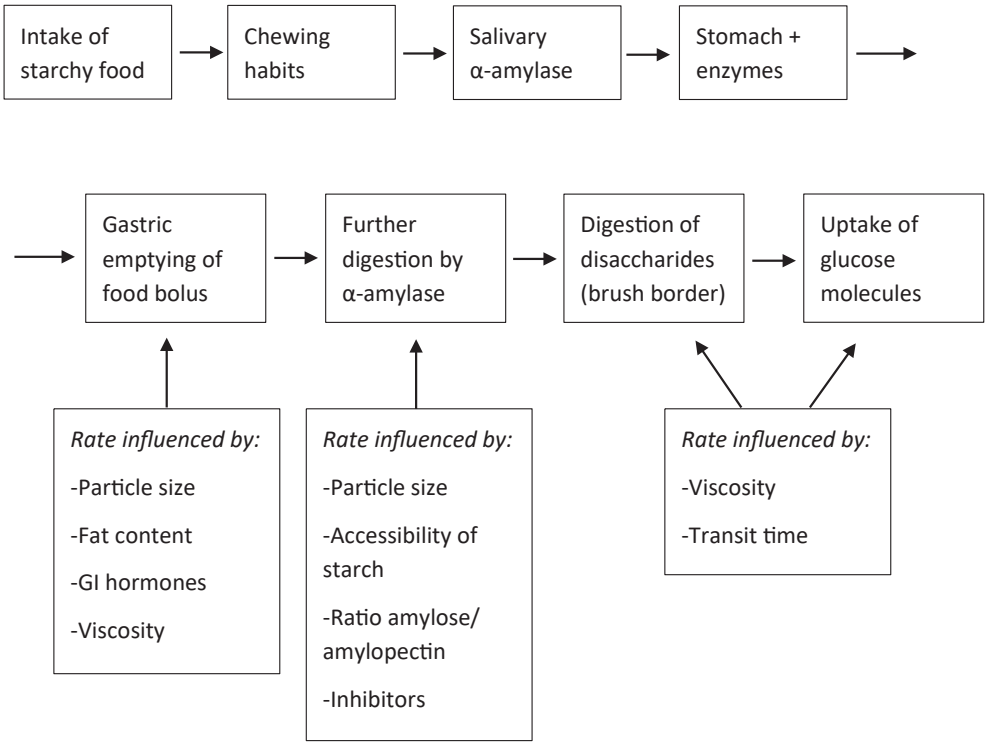

Figure 2. *Simplified schematic of factors involved in the rate of starch digestion and intestinal uptake of starch-derived glucose*

Techniques to study starch digestibility and/or uptake of starch-derived glucose

In vitro

Several *in vitro* methods exist to study starch and product-related characteristics which can give some information on starch digestibility and the effects of e.g. food processing. *In vitro* tests are product related, so characterizing the raw material or the end product.

Most widely used is the method by Englyst et al. (66), where starch is hydrolysed enzymatically with pancreatin and amyloglucosidases *in vitro*. Based on this method starch in foods may be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and RS. Another method to gain insight into the digestibility of the starch in the product is the hydrolysis index, which is an *in vitro* method measuring maltose after incubation with pepsin and pancreatic α-amylase. Because

food structure was increasingly being discussed as an important determinant of the metabolic responses to starchy foods, the test does include a person-related factor; mastication of the test product by volunteers (structure 'as eaten') is a first step (67). However, other physiological responses after the food intake (e.g. hormones, transit time, glucose uptake) are not taken into account. Several more sophisticated *in vitro* models are designed, imitating the entire gastro-intestinal tract (68).

The *in vitro* methods are useful to accurately study and compare starch digestibility of several products, and are also used to predict the glycemic response it will elicit in humans after consumption. However, hormonal responses regulating and influencing these processes *in vivo* cannot be mimicked in this way.

In vivo

With many nutritional studies in this area, where the focus is on the improvement of the glycemic response (via digestibility), different starchy food products are tested *in vivo* with the main outcome being the glycemic response or GI. In this way, the impact of the starchy food product on postprandial glycemia is known, taking into account the physiological processes involved. It is generally thought that the glycemic response or GI of a food product directly reflects the rate of starch digestion and entry of glucose into the systemic circulation. However, the total postprandial glycemic response is not only determined by starch digestibility and/or the rate of glucose absorption from the ingested meal. There is simultaneous clearance of glucose from the circulation due to mainly insulin-stimulated, but also insulin-independent, uptake of glucose into tissue, lowering the glycemic response. An example is a study by Schenk et al. where they show that the difference in GI of different breakfast cereals is not due to glucose influx from the meal, but due to a difference in glucose clearance (69). Endogenous glucose production, keeping glucose concentrations constant between meals (both glycogenolysis and gluconeogenesis), is suppressed postprandially, and should be taken into account as well (70).

Taken together, the glycemic response is the net result of these different post meal processes (**Figure 3**). To make a distinction between these three main processes contributing to total plasma glucose concentration and to get insight into the underlying postprandial glucose kinetics, stable isotopes can be used. These are isotopes of certain elements that naturally occur in a low abundance and do not decay. For instance, ^{13}C is a stable isotope of Carbon (^{12}C) with a natural abundance of around 1.08%. Some plants like corn, incorporate more ^{13}C in their tissue, leading to an abundance of 1.09%. The natural abundance of these isotopes can be increased by culturing plants in $^{13}CO_2$ -enriched atmosphere (71).

Administration of a starchy food product which is labeled with the stable isotope ^{13}C, enables the differentiation in plasma between exogenous glucose coming from the consumed test meal and endogenous glucose produced predominantly by the liver. By simultaneously infusing the volunteers with a known tracer amount of deuterium labeled glucose (D-[6,6-$^2H_2$]glucose), it is possible to monitor and correct for the uptake of glucose into the tissues, and calculate the glucose

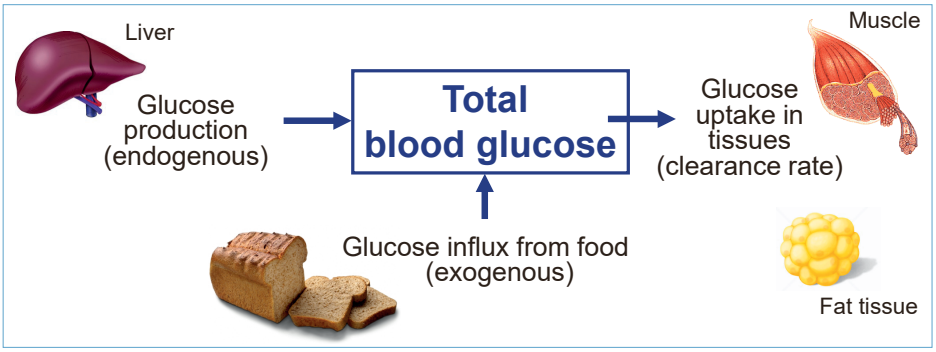

Figure 3. *Illustration of the different postprandial processes that result in the total blood glucose concentration.*

clearance rate (GCR). With the resulting data the 'Rate of appearance of Total glucose' (RaT) and the 'Rate of appearance of Exogenous glucose' (RaE) can be calculated, and by subtraction the endogenous glucose production (EGP) can be derived.

The RaE represents the systemic appearance of starch-derived glucose, and although it is measured (indirectly) after it has passed the liver, it is also seen as a reflection of influx of glucose from the intestine. It can give an indication of the digestibility of starch from the food product, and is therefore often referred to as '*in vivo* starch digestibility'. It must be noted that this is influenced by all processes related to starch digestion (including oral processing, gastric emptying, transit time, starch digestibility) *and* by the intestinal uptake of starch-derived glucose, thus reflecting the rate and extent of starch digestion and/or uptake of starch-derived glucose.

The different glucose fluxes can be related to relevant physiological factors such as the insulin response or concentrations of gastrointestinal hormones (section 1.3), which are influenced by and/or influence glucose kinetics. Thus, the use of this 'dual isotope technique' gives the ability to study starch- and product characteristics *in vivo* and to zoom in on the physiological effects of starch digestion and glucose uptake.

1.3 REGULATION OF POSTPRANDIAL GLUCOSE KINETICS AND METABOLISM

Several hormones are involved in the regulation of postprandial glucose kinetics and metabolism, being either released or suppressed in the postprandial state. Starch digestion and subsequent starch-derived glucose uptake affects the glucose and insulin response, as well as the release of some gastrointestinal hormones. Recently it has been found that other physiological factors, such as bile acids, may also play a role in glucose homeostasis. These factors related to the consumption of starchy food products will shortly be introduced in this section.

Insulin and glucagon

As indicated, the pancreatic hormones glucagon and insulin are important players in glucose homeostasis. They are produced and released by the α- and β- cells, respectively, located in the

islets of Langerhans, the endocrine part of the pancreas. By their opposing actions, they regulate glucose concentrations in the blood to keep it within the normal range. Where increasing glucagon levels stimulate the release and use of glucose from the internal glycogen stores (liver, muscle) and necessarily the synthesis of glucose (gluconeogenesis) between meals, the increase in insulin (mainly due to rising postprandial glucose levels) will inhibit these processes (72). Insulin also decreases the glucose levels by facilitating the uptake of glucose into cells of insulin sensitive tissues. By binding to the insulin receptor on muscle cells and adipocytes it regulates the translocation of intracellular vesicles containing GLUT4 to the plasma membrane. After fusion the GLUT4 transporters are inserted and become available for transporting glucose into the cells (73). Postprandial glucose uptake into tissue and thereby GCR is thus greatly determined by the action of insulin, whereas basal uptake of glucose via GLUT1 is a continuous process.

Glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1

The glucose-induced insulin response is greatly potentiated (up to 70%) by the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (74). Therefore, these incretin hormones play an important role in postprandial glucose homeostasis.

GIP is secreted from intestinal K-cells located in the duodenum, mainly in response to ingested glucose in a dose-dependent manner (75), but also triggered by proteins and lipids (76). In a previous study that used corn products, our group showed plasma GIP concentrations to correlate strongly with the RaE (77) and related GIP release to the rate of intestinal glucose uptake. The regulation of GLP-1 release from L-cells is less straight-forward, as more factors are involved. Nutrient ingestion is the main stimulus to L- cells, which are most abundantly present in the colon but also found in the duodenum in similar amounts as K-cells (78). The L-cells are described as an open type cell, enabling direct stimulation and release of GLP-1 by luminal contact (79). Other routes (via nerves or hormones) have also been proposed, but appear to play a less prominent role in humans (76).

Next to their role as an incretin, GIP and GLP-1 serve other functions as well (80). Studies have indicated that GIP is involved in several anabolic effects in adipose tissue, such as stimulation of fatty acid synthesis and enhancement of insulin-stimulated incorporation of fatty acids into triglycerides (81-83). GLP-1 reduces glucagon secretion (84, 85), decreases gastric emptying rate (86), induces satiety (87), and was found to restore β cell function in rodents (88). GLP-1 and related factors are also seen as important (pharmacological) targets in T2DM treatment and prevention.

Cholecystokinin and bile acids

Bile, which is continuously produced by the liver, is stored in the gall bladder and is released into the small intestine in response to food intake to aid in the digestive process. Gall bladder contraction is mainly stimulated via the intestinal hormone cholecystokinin (CCK), released from the duodenum in response to mainly fat and protein intake (89). CCK is also known for its inhibitory effects on gastric emptying and the induction of satiety (90).

Bile acids (BA) are known for their classical role in lipid metabolism, but receive more and more attention in relation to their function as signaling molecules in glucose metabolism. Bile acids are efficiently re-used after their presence in the intestine due to absorption by the enterocyte and transport back to the liver, known as the enterohepatic circulation. However, postprandial increases in plasma bile acids have been observed, indicated as spillover, which makes it possible to exert systemic effects as well (91). After an OGTT an increase in plasma bile acids was observed (92) at several time points postprandial (t = 0, 60 and 120) and also diurnal responses were described (93). Their actions also involve GLP-1 stimulation, making BAs a relevant factor in glucose homeostasis.

1.4 ABOUT THIS THESIS – SCOPE, AIM AND OUTLINE

Nutritional strategies for the prevention of the development of (pre-)diabetes may help to decrease the growing incidence of T2DM worldwide. Repeated high postprandial glucose concentrations in the blood are implicated with an increased risk on the development of metabolic disorders like T2DM. Starchy food products, such as bread and pasta, are widely consumed and the properties of these foods, such as the digestibility of starch, the presence of fiber or the food structure may play a role in the development or prevention of this disease. Therefore, a better understanding of their effects on physiology and metabolism and thereby their metabolic quality is necessary. This would also help in selecting the appropriate raw material and/or the best method of processing when designing beneficial starchy food products for the at risk population.

Aim and research questions

Thus, the aim of this thesis was to increase our insight into the metabolic effects of starchy foods in healthy men by studying the postprandial response and the glucose kinetics of a variety of wheat products differing in composition and/or structure.

The following research questions were addressed:

- What are the effects of product modification on the postprandial glucose response and underlying glucose kinetics?
- What are the effects of product modification on other postprandial metabolic factors like insulin, GIP, GLP-1, and bile acids?

Product modifications to bread included changes in bran type and kernel size, as well as changes in compactness of bread. High fiber wheat bread an pasta were used as control products. The common denominator in the described intervention studies was the use of stable isotopes (^{13}C and ^{2}H) to determine glucose kinetics (Chapter 1.2). Postprandial plasma concentrations of glucose, insulin, glucagon, and GIP were analyzed, and in a later stage also GLP-1, CCK and bile acids. This allowed linking '*in vivo* starch digestibility' to postprandial factors possibly related to the risk of development of T2DM. Additional metabolomics analyses of postprandial plasma samples was performed as well. A more detailed description of the study methods can be found in each separate chapter.

Outline of the thesis

In our first study, we aimed to investigate whether total plasma glucose concentrations reflect the *in vivo* starch digestibility of a starchy food product by comparing different ^{13}C-labeled wheat products (**Chapter 2**). A fiber-rich bread (control bread, CB1) was included as a product with an expected high glycemic response, whereas pasta with the same fiber content (PA1) was included as a product with an expected low glycemic response. Anthocyanins, a subclass of polyphenols, are capable of inhibiting α-amylase *in vitro* and are abundantly present in the fiber of purple wheat. In an attempt to decrease the glycemic response of a white wheat bread by slowing the rate of starch digestion, purple wheat bran was added.

Investigating the postprandial effects of bread and pasta (CB1 and PA1, Chapter 2), we would expect to see a high and a low glycemic response, respectively. However, as consumption of both fiber-rich wheat products resulted in a similar glycemic response, despite a clear difference in appearance of starch-derived glucose, we described in **Chapter 3** the more extended underlying glucose kinetics of this phenomenon, together with the postprandial responses of insulin and GIP.
In **Chapter 4**, a follow-up study was described investigating the effect of food structure on glucose kinetics and related postprandial variables. Based on the findings described in Chapter 3, we hypothesized that consumption of bread with a compact food structure would result in slower digestibility of starch, with an accompanying moderate metabolic response as seen for pasta, and a similar glycemic response compared to normal bread. Therefore, we designed compact flat breads (FB) without the use of a leavening agent and compared this to a yeast-leavened porous wheat bread (CB2, control bread with open structure) and to pasta (PA2, control wheat product with compact structure). All products had a similar composition, but different food structures due to the processing conditions.

Underlying mechanisms of the beneficial health effects of low glycemic index (GI) starchy foods are not fully elucidated yet. To be able to study the postprandial glucose kinetics of bread with a low glycemic response, we investigated in **Chapter 5** the effects of the substitution of 85% wheat flour by broken kernels (kernel bread, KB). Besides glucose kinetics, also postprandial insulin, glucagon, GIP, GLP-1, CCK and bile acid responses were studied.

Metabolomics was used to further deepen our insight into the acute metabolic consequences of the intake of products with a difference in appearance of starch-derived glucose, but a similar glycemic response. **Chapter 6** describes the metabolic profiles of nine healthy men that were analyzed after consumption of wheat bread (CB1) and pasta (PA1), both enriched with wheat-bran.
In **Chapter 7**, the results from these studies will be summarized and discussed based on the aforementioned research questions. This final chapter will be ended with describing the relevance of our findings for the prevention of type 2 diabetes, an overall conclusion and some future perspectives.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas. 8th edn. ed. Brussels, Belgium: 2017.
2. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC,Jr, Sowers JR. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 1999;100:1134-46.
3. Alberti G, Zimmet P, Shaw J, Bloomgarden Z, Kaufman F, Silink M, Consensus Workshop Group. Type 2 diabetes in the young: the evolving epidemic: the international diabetes federation consensus workshop. Diabetes Care 2004;27:1798-811.
4. Klein R. Hyperglycemia and microvascular and macrovascular disease in diabetes. Diabetes Care 1995;18:258-68.
5. Fu Z, R Gilbert E, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Current diabetes reviews 2013;9:25-53.
6. McDevitt RM, Bott SJ, Harding M, Coward WA, Bluck LJ, Prentice AM. De novo lipogenesis during controlled overfeeding with sucrose or glucose in lean and obese women. Am J Clin Nutr 2001;74:737-46.
7. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. Endocr Rev 2016;37:278-316.
8. Brownlee M. A radical explanation for glucose-induced beta cell dysfunction. J Clin Invest 2003;112:1788-90.
9. Levy J, Atkinson A, Bell P, McCance D, Hadden D. Beta-cell deterioration determines the onset and rate of progression of secondary dietary failure in type 2 diabetes mellitus: the 10-year follow-up of the Belfast Diet Study. Diabetic Med 1998;15:290-6.
10. Kahn SE. The importance of β-cell failure in the development and progression of type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism 2001;86:4047-58.
11. van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. Eur J Cardiovasc Prev Rehabil 2010;17 Suppl 1:S3-8.
12. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. The Lancet 2012;379:2279-90.
13. International Diabetes Federation. IDF Diabetes Atlas. 7th edn. ed. Brussels, Belgium: 2015.
14. Smyth S, Heron A. Diabetes and obesity: the twin epidemics. Nat Med 2006;12:75-80.
15. Tuso P. Prediabetes and lifestyle modification: time to prevent a preventable disease. Perm J 2014;18:88-93.
16. Roumen C, Corpeleijn E, Feskens E, Mensink M, Saris W, Blaak E. Impact of 3-year lifestyle intervention on postprandial glucose metabolism: The SLIM study. Diabetic Med 2008;25:597-605.
17. Penn L, White M, Lindström J, den Boer AT, Blaak E, Eriksson JG, Feskens E, Ilanne-Parikka P, Keinänen-Kiukaanniemi SM, Walker M. Importance of weight loss maintenance and risk prediction in the prevention of type 2 diabetes: analysis of European Diabetes Prevention Study RCT. PLoS One 2013;8:e57143.
18. Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. The Lancet 2014;383:1999-2007.
19. Hu FB, Van Dam R, Liu S. Diet and risk of type II diabetes: the role of types of fat and carbohydrate. Diabetologia 2001;44:805-17.
20. Jenkins DJA, Wolever TMS, Taylor RH. Glycemic index of foods: A physiological basis for carbohydrate exchange. Am J Clin Nutr 1981;34:362-6.
21. Wolever T. Is glycaemic index (GI) a valid measure of carbohydrate quality? Eur J Clin Nutr 2013;67:522-31.
22. Thomas D, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. Cochrane Database Syst Rev 2009;Art. No.: CD006296.
23. Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC.

Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care 1997;20:545-50.

24. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA 1997;277:472-7.
25. Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L, Hennekens CH, Manson JE. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. Am J Clin Nutr 2000;71:1455-61.
26. Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr 2004;80:348-56.
27. Schwingshackl L, Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutrition, Metabolism and Cardiovascular Diseases 2013;23:699-706.
28. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 2002;76:274S-80S.
29. Thomas D, Elliott EJ, Baur L. Low glycaemic index or low glycaemic load diets for overweight and obesity. Cochrane Database Syst Rev 2007;Art. No.: CD005105.
30. Hodge AM, English DR, O'Dea K, Giles GG. Glycemic index and dietary fiber and the risk of type 2 diabetes. Diabetes Care 2004;27:2701-6.
31. Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, Brand-Miller JC. Glycemic index, glycemic load, and chronic disease risk-a meta-analysis of observational studies. Am J Clin Nutr 2008;87:627-37.
32. Meyer KA, Kushi LH, Jacobs DR,Jr, Slavin J, Sellers TA, Folsom AR. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. Am J Clin Nutr 2000;71:921-30.
33. Sluijs I, Beulens JW, van der Schouw YT, van der ADL, Buckland G, Kuijsten A, Schulze MB, Amiano P, Ardanaz E, Balkau B, et al. Dietary glycemic index, glycemic load, and digestible carbohydrate intake are not associated with risk of type 2 diabetes in eight European countries. J Nutr 2013;143:93-9.
34. Blaak EE, Antoine JM, Benton D, Bjorck I, Bozzetto L, Brouns F, Diamant M, Dye L, Hulshof T, Holst JJ, et al. Impact of postprandial glycaemia on health and prevention of disease. Obes Rev 2012;13:923-84.
35. Livesey G, Taylor R, Hulshof T, Howlett J. Glycemic response and health--a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. Am J Clin Nutr 2008;87:258S-68S.
36. Chiasson J, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, STOP-NIDDM Trial Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. The Lancet 2002;359:2072-7.
37. Van de Laar, Floris A, Lucassen PL, Akkermans RP, Van de Lisdonk, Eloy H, De Grauw WJ. Alpha-glucosidase inhibitors for people with impaired glucose tolerance or impaired fasting blood glucose. Cochrane Database Syst Rev 2006;Art. No.: CD005061.
38. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care 2008;31:2281-3.
39. Biliaderis CG. The structure and interactions of starch with food constituents. Can J Physiol Pharmacol 1991;69:60-78.
40. Smith AM. The biosynthesis of starch granules. Biomacromolecules 2001;2:335-41.
41. Hoebler, M.-F. Devaux, A. Karinthi, C. Belleville, J.-L. Barry, C. Particle size of solid food after human mastication and *in vitro* simulation of oral breakdown. Int J Food Sci Nutr 2000;51:353-66.
42. Horowitz M, Dent J, Fraser R, Sun W, Hebbard G. Role and integration of mechanisms controlling gastric emptying. Dig Dis Sci 1994;39:7S-13S.
43. Dikeman CL, Fahey Jr GC. Viscosity as related to dietary fiber: a review. Crit Rev Food Sci Nutr 2006;46:649-

63.

44. Malagelada J, Azpiroz F. Determinants of gastric emptying and transit in the small intestine. Comprehensive Physiology 2011;909-37.
45. Caspary WF. Physiology and pathophysiology of intestinal absorption. The American Journal of Clinical Nutrition 1992;55:299S-308S.
46. Huge A, Weber E, Ehrlein H. Effects of enteral feedback inhibition on motility, luminal flow, and absorption of nutrients in proximal gut of minipigs. Dig Dis Sci 1995;40:1024-34.
47. Srichuwong S, Sunarti TC, Mishima T, Isono N, Hisamatsu M. Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. Carbohydr Polym 2005;60:529-38.
48. Srichuwong S, Sunarti TC, Mishima T, Isono N, Hisamatsu M. Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. Carbohydr Polym 2005;62:25-34.
49. Björck I, Granfeldt Y, Liljeberg H, Tovar J, Asp NG. Food properties affecting the digestion and absorption of carbohydrates. Am J Clin Nutr 1994;59:699S-705S.
50. Asp N, Björck I. Resistant starch. Trends Food Sci Technol 1992;3:111-4.
51. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81:1031-64.
52. Scazzina F, Del Rio D, Pellegrini N, Brighenti F. Sourdough bread: Starch digestibility and postprandial glycemic response. J Cereal Sci 2009;49:419-21.
53. Jenkins DJ, Wolever TM, Jenkins AL, Lee R, Wong GS, Josse R. Glycemic response to wheat products: reduced response to pasta but no effect of fiber. Diabetes Care 1983;6:155-9.
54. Granfeldt Y, Björck I, Hagander B. On the importance of processing conditions, product thickness and egg addition for the glycaemic and hormonal responses to pasta: A comparison with bread made from 'pasta ingredients'. Eur J Clin Nutr 1991;45:489-99.
55. Fardet A, Hoebler C, Baldwin P, Bouchet B, Gallant D, Barry J. Involvement of the Protein Network in the *in vitro* Degradation of Starch from Spaghetti and Lasagne: a Microscopic and Enzymic Study. J Cereal Sci 1998;27:133-45.
56. Jenkins DJA, Wesson V, Wolever TMS, Jenkins AL, Kalmusky J, Guidici S, Csima A, Josse RG, Wong GS. Wholemeal versus wholegrain breads: Proportion of whole or cracked grain and the glycaemic response. Br Med J 1988;297:958-60.
57. Liljeberg H, Granfeldt Y, Bjorck I. Metabolic responses to starch in bread containing intact kernels versus milled flour. Eur J Clin Nutr 1992;46:561-75.
58. Holt SHA, Brand Miller JC. Particle size, satiety and the glycaemic response. Eur J Clin Nutr 1994;48:496-502.
59. Jenkins DJA, Wolever TMS, Jenkins AL. Low glycemic response to traditionally processed wheat and rye products: Bulgur and pumpernickel bread. Am J Clin Nutr 1986;43:516-20.
60. Holm J, Bjorck I. Bioavailability of starch in various wheat-based bread products: Evaluation of metabolic responses in healthy subjects and rate and extent of *in vitro* starch digestion. Am J Clin Nutr 1992;55:420-9.
61. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem 2010;10:315-31.
62. Coe S, Ryan L. Impact of polyphenol-rich sources on acute postprandial glycaemia: a systematic review. Journal of Nutritional Science 2016;5:e24.
63. Barrett ML, Udani JK. A proprietary alpha-amylase inhibitor from white bean (Phaseolus vulgaris): a review of clinical studies on weight loss and glycemic control. Nutrition Journal 2011;10:24.
64. Collier G, O'Dea K. The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein. Am J Clin Nutr 1983;37:941-4.

65. Liljeberg HG, Bjorck IM. Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: studies in humans and rats using test products with added organic acids or an organic salt. Am J Clin Nutr 1996;64:886-93.

66. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH. Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. Am J Clin Nutr 1999;69:448-54.

67. Granfeldt Y, Bjorck I, Drews A, Tovar J. An *in vitro* procedure based on chewing to predict metabolic response to starch in cereal and legume products. Eur J Clin Nutr 1992;46:649-60.

68. Minekus M, Marteau P, Havenaar R, Huis in 't Veld, J.H.J. Multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. Alternatives to laboratory animals: ATLA 1995;23:197-209.

69. Schenk S, Davidson CJ, Zderic TW, Byerley LO, Coyle EF. Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am J Clin Nutr 2003;78:742-8.

70. Priebe MG, Wachters-Hagedoorn RE, Heimweg JA, Small A, Preston T, Elzinga H, Stellaard F, Vonk RJ. An explorative study of *in vivo* digestive starch characteristics and postprandial glucose kinetics of wholemeal wheat bread. Eur J Nutr 2008;47:417-23.

71. Verbeke K, Ferchaud-Roucher V, Preston T, Small AC, Henckaerts L, Krempf M, Wang H, Vonk RJ, Priebe MG. Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers. Eur J Clin Nutr 2010;64:678-84.

72. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectrum 2004;17:183-90.

73. Cushman SW, Wardzala LJ. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem 1980;255:4758-62.

74. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 1986;63:492-8.

75. Schirra J, Katschinski M, Weidmann C, Schafer T, Wank U, Arnold R, Goke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 1996;97:92-103.

76. Deacon CF, Ahren B. Physiology of incretins in health and disease. Rev Diabet Stud 2011;8:293-306.

77. Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Englyst KN, Holst JJ, Stellaard F, Vonk RJ. The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic polypeptide concentrations in healthy men. J Nutr 2006;136:1511-6.

78. Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM. Human duodenal enteroendocrine cells: Source of both incretin peptides, GLP-1 and GIP. American Journal of Physiology - Endocrinology and Metabolism 2006;290:E550-9.

79. Eissele R, Goke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Goke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 1992;22:283-91.

80. Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131-57.

81. Yip RGC, Wolfe MM. GIP biology and fat metabolism. Life Sci 2000;66:91-103.

82. Song DH, Wolfe MM. Glucose-dependent insulinotropic polypeptide and its role in obesity. Curr Opin in Endocrinol Diabetes Obes 2007;14:46-51.

83. Paschetta E, Hvalryg M, Musso G. Glucose-dependent insulinotropic polypeptide: from pathophysiology to therapeutic opportunities in obesity-associated disorders. Obes Rev 2011;12:813-28.

84. Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, Tarui S. Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7-36)-amide. Diabetes 1989;38:902-5.

85. Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia

by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993;36:741-4.

86. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Ørskov C, Ritzel R, Schmiegel WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 1997;273:E981-8.
87. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 1998;101:515-20.
88. Drucker DJ. Glucagon-like peptide-1 and the islet beta-cell: augmentation of cell proliferation and inhibition of apoptosis. Endocrinology 2003;144:5145-8.
89. Hopman W, Jansen J, Lamers C. Comparative study of the effects of equal amounts of fat, protein, and starch on plasma cholecystokinin in man. Scand J Gastroenterol 1985;20:843-7.
90. Moran TH, McHugh PR. Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am J Physiol 1982;242:R491-7.
91. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nature reviews Drug discovery 2008;7:678-93.
92. Matysik S, Martin J, Bala M, Scherer M, Schaffler A, Schmitz G. Bile acid signaling after an oral glucose tolerance test. Chem Phys Lipids 2011;164:525-9.
93. Steiner C, Othman A, Saely CH, Rein P, Drexel H, von Eckardstein A, Rentsch KM. Bile acid metabolites in serum: intraindividual variation and associations with coronary heart disease, metabolic syndrome and diabetes mellitus. PLoS One 2011;6:e25006.

The glycemic response does not reflect the *in vivo* starch digestibility of fiber-rich wheat products in healthy men

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ABSTRACT

Starchy food products differ in the rate of starch digestion which can affect their metabolic impact. In this study we examined how the *in vivo* starch digestibility is reflected by the glycemic response because this response is often used to predict starch digestibility. Ten healthy male volunteers (age 21 ± 0.5 y, BMI 23 ± 0.6 kg/m^2 [mean ± SEM]) participated in a cross-over study, receiving three different meals: pasta with normal wheat bran (PA) and bread with normal (CB) or purple wheat bran (PBB). Purple wheat bran was added in an attempt to decrease the rate of starch digestion. The meals were enriched in ^{13}C and the dual isotope technique was applied in order to calculate the rate of appearance of exogenous glucose (RaE). The ^{13}C-isotopic enrichment of glucose in plasma was measured with gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) and liquid chromatography (LC)/IRMS. Both IRMS techniques gave similar results. Plasma glucose concentrations (2 h incremental area under the curve (iAUC)) were not different between the test meals. The RaE was similar after consumption of CB and PBB, showing that purple wheat bran in bread does not affect *in vivo* starch digestibility. However, the incremental AUC of RaE after men consumed PA was less than after they consumed CB ($p<0.0001$), despite the similar glucose response. To conclude, the glycemic response does not always reflect the *in vivo* starch digestibility. This could have implications for intervention studies in which the glycemic response is used to characterize test products.

INTRODUCTION

Starch digestibility, which varies widely between different food products, is implicated in the pathogenesis, treatment and prevention of chronic diseases. Frequent consumption of rapidly digestible starch, resulting in postprandial hyperglycemia and -insulinemia, might be a risk factor for the development of insulin resistance, T2DM and cardiovascular (1-3). For T2DM-patients it is important to avoid postprandial hyperglycemia, and foods with slowly digestible carbohydrates can contribute to improved glycemic control (4). Slowly digestible starch might also be beneficial due to the increased nutrient delivery to the distal small intestine and the resulting increase in plasma GLP-1 concentrations, which is one of the proposed explanations for the complete remission of T2DM after bariatric surgery (5). In addition, fermentation of starch that reaches the colon increases the production of short-chain fatty acids which are associated with several health benefits (6,7).

Information about starch digestibility can be obtained from *in vitro* assays (8) which, however, might not always predict the *in vivo* starch digestion as we have shown before (9). In addition, carbohydrate-rich foods are often classified using the GI, which reflects the effect on postprandial blood glucose concentrations (10). A low GI of starchy foods is generally assumed to be due to slow digestibility of starch. However, the postprandial glucose response is not only determined by the glucose absorbed from the ingested meal. Endogenous glucose production and glucose uptake into tissues contribute to the postprandial glucose concentrations measured. To get insight into the *in vivo* digestive behavior of starchy foods and how this relates to the glycemic response, stable isotopes can be used. By labeling the glucose in the food products with ^{13}C and infusing the volunteer with a tracer amount of D-[6,6-$^{2}H_{2}$]glucose the above mentioned processes contributing to total glucose concentration can be distinguished. So far, only little data is available describing the value of the glycemic response of different starchy foods for predicting starch digestibility. With this study we aim to extent this knowledge by investigating different wheat products.

One of the most common sources of starch in human nutrition is wheat (*Triticum* spp), and products made from wheat can be either high or low glycemic. In general, the consumption of wheat bread results in high postprandial glucose concentrations (high GI) (11) and the starch in wheat bread is therefore considered to be rapidly digested. A wheat product such as pasta is considered as more slowly digestible, due to a generally lower, more prolonged elevation of postprandial glucose concentrations (low GI) (11). Considering the negative aspects of a diet rich in rapidly digestible starch several attempts are being made to diminish the rapid hydrolysis of starch, for instance by inhibiting the digestive enzymes using natural ingredients (12). Anthocyanins, a subgroup of plant-derived polyphenols, have been shown to inhibit the action of α-amylase and/or α-glucosidase *in vitro (*13-18). Besides their presence in berries, vegetables and red wine, purple wheat (*T. aethiopicum*) has high anthocyanin concentrations in the pericarp (bran fraction) (19, 20). Thus, we hypothesized that the addition of purple wheat bran decreases the digestion rate of rapidly digestible wheat bread.

In order to calculate the rate of starch digestion and uptake of starch derived glucose *in vivo*

various methods can be used to measure isotopic enrichment of plasma glucose. In our studies ^{13}C-isotopic enrichment is usually determined with GC/C/IRMS, requiring derivatization of plasma samples prior to analysis (21-24). Since the introduction of the LC interface for IRMS by Krummen et al. (25) several studies showed the power and accuracy of this method in many disciplines (26-30). As the method using GC/C/IRMS is a labor-intensive method which might increase the uncertainty of the measured isotopic composition, Schierbeek et al. (31) developed a more simple method for the simultaneous measurement of ^{13}C-glucose enrichment and glucose concentration in human plasma, using LC/IRMS. In the present study this method was used in addition to our standard GC/C/IRMS method.

In summary, this study aimed to investigate whether total plasma glucose concentrations reflect the *in vivo* starch digestibility of a starchy food product by comparing different ^{13}C-labeled wheat products (breads and pasta). In an attempt to decrease the rate of starch digestion, wheat bread was made with added purple wheat bran. To evaluate our results concerning ^{13}C-enrichment of glucose in plasma obtained with GC/C/IRMS a comparison was made with LC/IRMS-data.

PARTICIPANTS AND METHODS

Participants

Ten healthy men (age 21 ± 0.5 y, BMI 23 ± 0.6 kg/m^2 [mean ± SEM]) were recruited. Criteria for exclusion were use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the BEBO foundation, Assen, The Netherlands. Each participant gave written informed consent for the study.

Experimental design

The study was performed in a crossover manner, with each participant studied on three occasions at least one week apart. The participants refrained from consuming ^{13}C-enriched foods like cane sugar, corn products and pineapple, for three days and from alcohol consumption and strenuous exercise for 24 h before each study day. Food intake on the day before each experiment was individually standardized using a diary. A standard evening meal was provided at the research facility, where the men stayed overnight. Participants fasted overnight, but were allowed to drink water. A venous catheter was inserted in each forearm: for blood collection and for infusion of D-[6,6-$^2H_2$]glucose (98% ^2H APE) (Isotec Inc, Miamisburg, OH, USA). In the morning (t=-120) 26.7 mL D-[6,6-$^2H_2$]glucose solution (80 × 0.07 mg/kg body weight) was infused, and a continuous infusion of 0.07 mg/kg body weight D-[6,6-$^2H_2$]glucose per min was started and maintained for 8 h. Two hours after the start of the infusion (t= 0) the test meal was ingested. During the study period physical activity was limited.

Sample collection

Blood was collected into 2 mL BD Vacutainer Fluoride tubes (BD Diagnostics, Franklin Lakes, NJ).

Three basal blood samples were collected (t= -60, t= -30, t= -5) and after the test meal samples were drawn every 15 min for 2 h and every 30 min for an additional 4 h. After centrifugation (1300 × *g* for 10 min at 4 °C), sample aliquots were stored at -20 °C until analysis.

Test meals and preparation

The three wheat-based test meals were 132 g control bread with normal wheat bran (CB), 132 g bread with purple wheat bran (PBB) and 119 g (uncooked weight) pasta with normal wheat bran (PA), all prepared at TNO Healthy Living, Zeist, The Netherlands. Each test meal consisted of 50 g available carbohydrates and was consumed together with 10 g margarine light (4 g fat), 2 slices lean ham (5 g fat, 6 g protein) and 250 mL tap water within 20 min.

Meals were ^{13}C-enriched by the addition of 12% ^{13}C-labeled wheat (*T. aestivum* var *Paragon* (1.359 AP ^{13}C)) cultured in a $^{13}CO_2$ enriched atmosphere, as described before (32). Normal wheat bran (English Biscuit Wheat) and purple wheat bran (Purple Wheat) were purchased from Meneba, Rotterdam, The Netherlands.

Breads were prepared with 1110 g unlabelled white wheat flour (*T. aestivum* Kolibri/Ibis (ratio 70/30)) (1.085 AP ^{13}C), 210 g wheat bran (normal or purple bran, resulting in 7% dietary fiber in the final product), 180 g ^{13}C-labeled wheat flour, 975 g water, 25 g yeast and 30 g salt. After kneading, the dough was let to rise for 30 min, and was baked for 30 min at 240 °C. Portions of 132 g bread were stored at -20 °C until use. PA was freshly prepared with 739 g wheat flour (*T. durum* de Cecco), 141 g normal wheat bran and 120 g ^{13}C-labeled wheat flour, 400 g water and 20 g salt. Portions of 119 g were stored at 5 °C until use and cooked for 6.5 min in 2 L water before consumption.

Determination of total phenolic concentration

Polyphenols were extracted from the bran and the test meals by boiling in water under reflux (30 min). The total phenolic concentration of the extract was determined using the Folin-Ciocalteu method (limit of detection 0.1g/100g) as described by Singleton et al. (33).

***In vitro* analysis of starch fractions**

In order to analyse the starch fractions (G_T, G_{RA}, G_{SA}, G_{TA} and RS) in the test meals, an adapted version of the Englyst method (8) was used, as described previously (32).

To determine the ^{13}C abundance of the test meals, necessary for further calculations, the glucose resulting from this method was derivatized (as described later for plasma samples) and measured by GC/C/IRMS.

Measurement of plasma glucose concentrations

Plasma glucose concentrations were measured on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi, Tokyo, Japan) using a glucose hexokinase method. The within- and between-run CV were ≤ 2 %.

Analysis of isotopic enrichment by GC/MS and GC/C/IRMS

Analysis of isotopic enrichment in plasma by GC requires the derivatization of glucose to glucose penta acetate. The sample preparation was described in detail elsewhere (23, 24).

^{2}H enrichment was measured by GC/MS as described previously (23), with some modifications. The GC/MS system used was an Agilent 5975 MSD quadrupole instrument (Agilent Technologies, Amstelveen, The Netherlands). The sample was separated on a 30 m × 0.25 mm (0.25 µm film thickness) AT 1701 capillary column (Alltech Ass. Inc., Deerfield, IL, USA). The initial column temperature was 150 °C for 0.5 min and was increased to 280 °C (80 °C/min). The MS was used in the chemical ionization mode and selected ion monitoring was performed for the mass ions *m/z* 331 (M0) and 333 (M2).

$^{13}C/^{12}C$ isotope ratio was measured using GC/C/IRMS as described previously (21, 22), with some modifications. A Delta plus XL HP 6890 series GC system (Finnigan MAT, Bremen, Germany) was used. The sample was injected in the splitless mode onto a 30 m × 0.32 mm (0.25 µm film thickness) J&W Scientific column (Folsom, CA, USA). Eluting compounds were combusted on-line in a platin catalyzed CuO oxidation reactor operating at 940 °C.

Analysis of isotopic enrichment by LC/IRMS

An aliquot plasma (25 µL) was mixed with 50 µL of 1 mmol/L trehalose (internal standard) and deproteinated by adding 125 µL of 2 mol/L perchloric acid, incubating for 10 min on ice and centrifugation at 10,000 × *g* for 20 min. The supernatant was filtered through 0.2 µm Nylon membrane filters (Grace Alltech, Breda, Netherlands). The injection volume was 20 µL.

High performance ion-exclusion chromatography was carried out on a LC system consisting of two Knauer pumps (Berlin, Germany) and a Midas auto sampler (Spark, Emmen, The Netherlands), fitted with a Transgenomic ICSep ION-300 column (300 × 7.8 mm) (Achrom, Zulte, Belgium). The LC system was coupled to the IRMS instrument by a LC-Isolink interface (Thermo Fisher, Bremen, Germany), which is based on wet oxidation of organic components with sodium peroxodisulfate under acidic conditions. The flow rates of the acid reagent (1.5 mol/L phosphoric acid) and the oxidant reagent (0.84 mol/L peroxodisulfate) in the LC interface were both 25 µL/min. Isotopic ratio measurements were carried out on a Delta XP IRMS instrument (Thermo Fisher, Bremen, Germany). The ion source pressure was 0.4 mPa, and ions were generated by electron impact at 70 eV. The CO_2^+ signals for the three major ions at *m/z* 44 ($^{12}CO_2$), *m/z* 45 ($^{13}CO_2$ and $^{12}C^{17}O^{16}O$) and *m/z* 46 ($^{12}C^{18}O^{16}O$) were monitored.

Calculations

The $^{13}C/^{12}C$ abundance ratio was expressed as $\delta\ ^{13}C$ (‰) value. The delta notation is defined as $\delta\ ^{13}C_{sample} = [(R_s / R_{st}) - 1] \times 1000$, where R_s is the $^{13}C/^{12}C$ ratio in the sample and R_{st} is the $^{13}C/^{12}C$ ratio of the International Standard of Vienna Pee Dee Belemnite. The $\delta\ ^{13}C$ was transformed to the abundance of ^{13}C in a sample, defined as AP, as follows:

$$AP = Atom\ \% = \left[\frac{100 \times R_{st} \times \left(\left(\delta^{13}C/1000\right)+1\right)}{1+R_{st} \times \left(\left(\delta^{13}C/1000\right)+1\right)}\right]$$

The AP values retrieved from GC/C/IRMS measurement represent the ^{13}C abundance of glucose penta acetate. In order to calculate the AP from glucose, the AP from penta acetate was determined using two reference glucose samples (from corn and potato) as described previously (23). The obtained value was used to correct for derivatization of the plasma glucose. APE, the isotopic enrichment in a sample compared to the basal value, is defined as AP (sample) minus AP (natural abundance).

The systemic RaE was calculated as described by Tissot et al. (34). The used molar percentage enrichment of [6,6-$^{2}H_{2}$]glucose (calculated as previously described (23)) and the AP curves were first smoothed (35) before further calculations.

To determine differences in plasma glucose concentrations and RaE, the iAUC was calculated as described before (24) and compared for the time period 0–120 min.

Statistics

Data are presented as means ± SEM. A mixed model was used to test statistical differences between meals within participants, using test meal as a fixed factor and participant as a random factor. Partial tests were corrected for multiple comparison (Dunnet, with CB as reference). All analyses were performed with the software package SAS (release 9.1; SAS Institute Inc., Cary, NC). A *P* value <0.05 was considered statistically significant.

RESULTS

Characterization of test meals

The ^{13}C abundance of CB and PBB was 1.118 AP ^{13}C and was 1.119 AP ^{13}C in the PA.

A clear difference in total polyphenol concentration between normal (0.58 g/100 g bran) and purple bran (0.96 g/100 g bran) was measured, however, no difference could be measured between both breads (CB and PBB: 0.10 g/100 g bread), possibly due to the sensitivity of the method.

In vitro analysis of starch fractions showed only minor differences in the G_{RA} fraction of the meals (**Table 1**). The percentage G_{SA} found in PA was almost double the percentage determined for both breads.

Postprandial plasma glucose concentrations

Concerning PA, data is available from 9 participants as one did not consume the entire meal. Fasting plasma glucose concentrations did not differ between study days (*P*=0.68), nor did the peak values (*P*=0.35) (**Figure 1A**). The iAUC were not different after men consumed CB (144 ± 15.9 mmol/L·2h), PBB (168 ± 32.0 mmol/L·2h) and PA (115 ± 20.6 mmol/L·2h) (*P*=0.11).

Table 1. *In vitro analysis of starch fractions in the test meals*[1]

	Test meal		
Starch fraction	CB	PBB	PA
		%	
G_T	100	100	100
G_{RA}	91.4	90.3	87.0
G_{SA}	6.1	5.4	10.6
G_{TA}	97.5	95.7	97.6
RS	2.3	3.9	2.2

[1]CB, control bread with normal wheat bran; G_{RA}, rapidly available glucose (20 min); G_{SA}, slowly available glucose (20-120 min); G_T, total glucose (indicated as 100%); G_{TA}, total available glucose (120 min); PA, pasta with normal wheat bran, PBB, bread with purple wheat bran, RS, resistant starch.

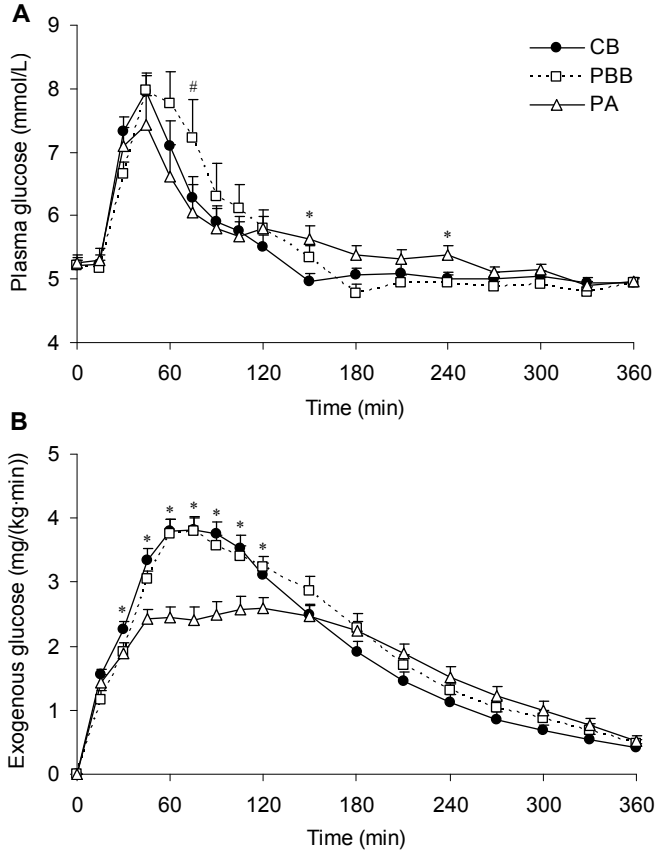

Figure 1. *Postprandial plasma glucose concentrations (A) and rate of appearance of exogenous glucose concentrations (RaE) (B) in healthy men after ingestion of 132 g ^{13}C-enriched control bread with normal wheat bran (CB), 132 g ^{13}C-enriched bread with purple wheat bran (PBB) and 119 g (uncooked weight) ^{13}C-enriched pasta (PA). Values are means ± SEM, n=10 (n=9 for PA). *PA is different from CB (P<0.05), #PBB is different from CB (P<0.05).*

RaE

The rate at which glucose from both breads appeared in the systemic circulation showed no difference, whereas after the consumption of PA it was much slower (**Figure 1B**). The iAUC of RaE after men consumed PA (37 ± 1.5 %dose·2h) was less than after they consumed CB (54 ± 2.9 %dose·2h) (P<0.0001).

^{13}C-isotopic enrichment measured with GC/C/IRMS and LC/IRMS

Comparison of APE obtained with LC/IRMS and GC/C/IRMS showed only minor deviations (**Supplemental Figure 1**). The agreement between both methods was also visualized using a Bland-Altman plot (**Figure 2A**). in which the majority of points were within the 95% limit of agreement

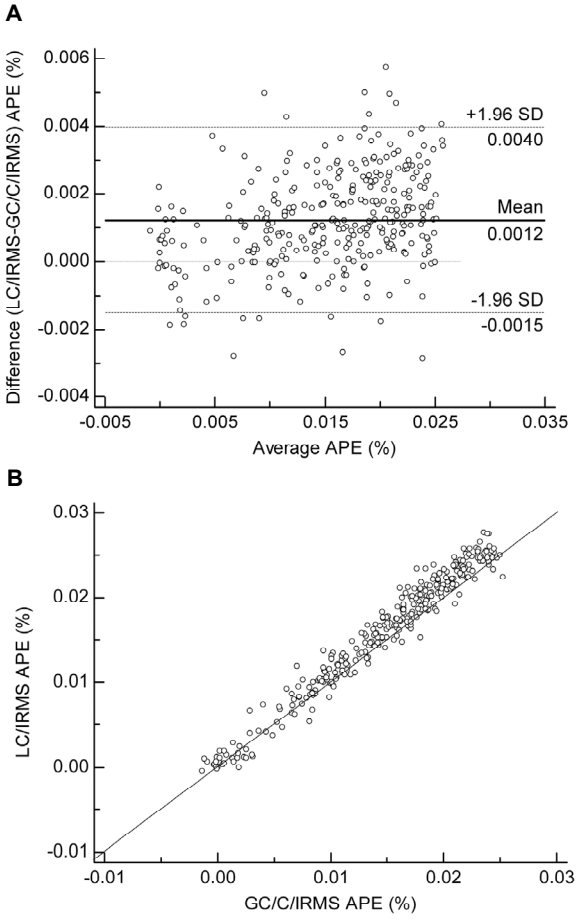

Figure 2. *Comparison of ^{13}C Atom Percent Excess (APE) values determined with gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) and liquid chromatography (LC)/IRMS in 11 postprandial plasma samples from 10 healthy men after ingestion of three ^{13}C-enriched test meals visualized in a Bland-Altman plot (A) and a scatter plot (B) (n=328 (33 in each man, 2 missing values)).*

(-0.0015 to 0.0040 %). The mean difference between both methods was 0.0012 %, indicating that the APE values calculated from the measurements on the LC/IRMS were slightly higher. This also appeared from the scatter plot (**Figure 2B**) where, especially in the higher region, the points were mainly above the line of equality.

DISCUSSION

This study aimed to investigate to what extent the glycemic response reflects the *in vivo* starch digestibility of a starchy food product, by comparing different ^{13}C-labeled wheat products. Comparing the iAUC of postprandial glucose concentrations after men consumed PA and CB, no difference was observed, whereas a pronounced difference was found between the RaE after consumption of these test meals. While the RaE after men consumed both breads was comparable, this was much slower after they consumed PA, with the iAUC being 30% smaller as compared to CB. Because the glycemic response was unable to show the slowly digestible properties of PA, these results indicate that total postprandial glucose concentrations do not always predict the *in vivo* digestion rate of a starchy food product. This could be explained by various factors, as for example a difference in endogenous glucose production or glucose clearance rate, which will be examined in detail in future work. In a similar study Schenk et al. (36) investigated the underlying glucose kinetics of breakfasts with a low and a high GI. Despite a pronounced difference in total blood glucose response, the RaE was similar for both test meals. The observed low glycemic response was explained by a difference in tissue glucose uptake. The discrepancy between the glycemic response and RaE observed by both Schenk et al. and us emphasize the importance of determining RaE by using stable isotope technology. This is especially relevant in mechanistic studies relating intestinal absorption of starch derived glucose with metabolic effects. Also, intervention studies investigating the effect of a low versus high GI diet might give more consistent results when the test products are characterized based on the RaE rather than the glycemic response.

A bread with purple wheat bran was made in an attempt to slow starch digestion, because the principal anthocyanin present in purple wheat, cyanidin-3-glucoside (19, 20), has shown inhibitory activity towards pancreatic α-amylase *in vitro (*18). However, in our study the purple wheat bran in the wheat bread had no effect on the rate of starch digestion. Recently, the addition of polyphenol-rich berries to a starch-based food product was also reported to have no effect on the glycemic response (37).

Total polyphenol concentrations in the purple wheat bran were higher than in normal wheat bran, as expected due to higher anthocyanin concentrations in the pericarp of purple wheat (19, 20). However, this difference could not be measured in CB and PBB, possibly because in the complete breads the polyphenol concentrations were around the limit of detection of the method due to 'dilution' of the bran fraction with other ingredients. Baking of the bread might also have had an effect on polyphenol concentrations, as anthocyanin pigments readily degrade during thermal processing (38). Li et al. (39) showed that the antioxidant capacity of heat-treated purple wheat

bran was not diminished, but whether this holds true for the amylase-inhibiting properties of the anthocyanins after baking is not known.

The ^{13}C-glucose measurement with GC/C/IRMS requires the derivatization of glucose to glucose penta acetate, which is a laborious procedure. To obtain the degree of isotopic enrichment of glucose without the penta acetate ester a correction is needed, which might increase the uncertainty of the measured isotopic composition. Therefore, the ^{13}C enrichment in postprandial plasma samples was also determined using a recent developed LC/IRMS method (31), which does not need derivatization of glucose. Comparison of all data indicated that LC/IRMS measurements result in a slightly higher APE, mainly in the higher APE region. As in this study the GC/C/IRMS data was used for RaE calculation, no overestimation of differences between bread and pasta was made. Taken together, the comparison of both analytical methods confirmed and strengthened our findings. Determination of ^{13}C enrichment in plasma using LC/IRMS is however more convenient due to less laborious sample preparation and therefore recommended as method of choice. In our dual label studies derivatization is also required for ^{2}H-glucose determination by GC/MS, making the use of GC/C/IRMS for determination of ^{13}C/^{12}C ratio more practical. Thus, the use of stable isotopes is a robust method providing good insight in the *in vivo* rate of starch digestion. Both GC/C/IRMS and LC/IRMS techniques provided reliable results enabling the calculation of RaE from each test meal.

In conclusion, this study showed that the *in vivo* rate of starch digestion can not always be predicted by measuring the glycemic response. Despite a similar glycemic response after men consumed CB and PA, the RaE was much slower after they consumed PA. In order to generate reliable and comparable results in intervention studies investigating low vs high GI diets, care should be taken when characterizing test meals based on their GI. Ingestion of two products with the same GI can - due to different glucose kinetics - result in different postprandial metabolic processes which consequently can lead to great variances in the outcome measures under study.

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REFERENCES

1. Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L, Hennekens CH, Manson JE. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. Am J Clin Nutr. 2000;71:1455-61.
2. Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care. 1997;20:545-50.
3. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA. 1997;277:472-7.
4. Thomas D, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. Cochrane Database Syst Rev. 2009;21:Art. No.: CD006296.
5. Thaler JP, Cummings DE. Hormonal and metabolic mechanisms of diabetes remission after gastrointestinal surgery. Endocrinology. 2009;150:2518-25.
6. Meijer K, De Vos P, Priebe MG. Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? Curr Opin Clin Nutr Metab Care. 2010;13:715-21.
7. Wong JMW, De Souza R, Kendall CWC, Emam A, Jenkins DJA. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol. 2006;40:235-43.
8. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH. Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. Am J Clin Nutr. 1999;69:448-54.
9. Priebe MG, Wachters-Hagedoorn RE, Heimweg JA, Small A, Preston T, Elzinga H, Stellaard F, Vonk RJ. An explorative study of *in vivo* digestive starch characteristics and postprandial glucose kinetics of wholemeal wheat bread. Eur J Nutr. 2008;47:417-23.
10. Jenkins DJA, Wolever TMS, Taylor RH. Glycemic index of foods: A physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981;34:362-6.
11. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care. 2008;31:2281-3.
12. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem. 2010;10:315-31.
13. Adisakwattana S, Ngamrojanavanich N, Kalampakorn K, Tiravanit W, Roengsumran S, Yibchok-Anun S. Inhibitory activity of cyanidin-3-rutinoside on α-glucosidase. J Enzyme Inhib Med Chem. 2004;19:313-6.
14. Adisakwattana S, Charoenlertkul P, Yibchok-Anun S. α-glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose. J Enzyme Inhib Med Chem. 2009;24:65-9.
15. Iwai K, Kim MY, Onodera A, Matsue H. α-Glucosidase inhibitory and antihyperglycemic effects of polyphenols in the fruit of Viburnum dilatatum thunb. J Agric Food Chem. 2006;54:4588-92.
16. Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K. α-glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. J Agric Food Chem. 2001;49:1948-51.
17. McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. J Agric Food Chem. 2005;53:2760-6.
18. Akkarachiyasit S, Charoenlertkul P, Yibchok-Anun S, Adisakwattana S. Inhibitory activities of cyanidin and its glycosides and synergistic effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase. Int J Mol Sci. 2010;11:3387-96.
19. Abdel-Aal ESM, Hucl P. Composition and stability of anthocyanins in blue-grained wheat. J Agric Food Chem. 2003;51:2174-80.
20. Hosseinian FS, Li W, Beta T. Measurement of anthocyanins and other phytochemicals in purple wheat. Food Chem. 2008;109:916-24.

21. Vonk RJ, Hagedoorn RE, De Graaff R, Elzinga H, Tabak S, Yang YX, Stellaard F. Digestion of so-called resistant starch sources in the human small intestine. Am J Clin Nutr. 2000;72:432-8.

22. Vonk RJ, Lin Y, Koetse HA, Huang CY, Zeng G, Elzinga H, Antoine JM, Stellaard F. Lactose (mal)digestion evaluated by the ^{13}C-lactose digestion test. Eur J Clin Invest. 2000;30:140-6.

23. Vonk RJ, Stellaard F, Priebe MG, Koetse HA, Hagedoorn RE, De BS, Elzinga H, Lenoir-Wijnkoop I, Antoine JM. The ^{13}C/^{2}H-glucose test for determination of small intestinal lactase activity. Eur J Clin Invest. 2001;31:226-33.

24. Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Englyst KN, Holst JJ, Stellaard F, Vonk RJ. The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic polypeptide concentrations in healthy men. J Nutr. 2006;136:1511-6.

25. Krummen M, Hilkert AW, Juchelka D, Duhr A, Schlüter HJ, Pesch R. A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2004;18:2260-6.

26. Boschker HTS, Moerdijk-Poortvliet TCW, Van Breugel P, Houtekamer M, Middelburg JJ. A versatile method for stable carbon isotope analysis of carbohydrates by high-performance liquid chromatography/isotope ratio mass spectrometry. Rapid Commun Mass Spectrom. 2008;22:3902-8.

27. McCullagh J, Gaye-Siessegger J, Focken U. Determination of underivatized amino acid δ^{13}C by liquid chromatography/isotope ratio mass spectrometry for nutritional studies: The effect of dietary non-essential amino acid profile on the isotopic signature of individual amino acids in fish. Rapid Commun Mass Spectrom. 2008;22:1817-22.

28. Schierbeek H, Te Braake F, Godin JP, Fay LB, Van Goudoever JB. Novel method for measurement of glutathione kinetics in neonates using liquid chromatography coupled to isotope ratio mass spectrometry. Rapid Commun Mass Spectrom. 2007;21:2805-12.

29. Schierbeek H, Rook D, Te Braake FWJ, Dorst KY, Voortman G, Godin JP, Fay LB, Goudoever JBV. Simultaneous analysis of ^{13}C-glutathione as its dimeric form GSSG and its precursor [1-^{13}C]glycine using liquid chromatography/isotope ratio mass spectrometry. Rapid Commun Mass Spectrom. 2009;23:2897-902.

30. Smith CI, Fuller BT, Choy K, Richards MP. A three-phase liquid chromatographic method for δ^{13}C analysis of amino acids from biological protein hydrolysates using liquid chromatography-isotope ratio mass spectrometry. Anal Biochem. 2009;390:165-72.

31. Schierbeek H, Moerdijk-Poortvliet TCW, Van Den Akker CHP, Te Braake FWJ, Boschker HTS, Van Goudoever JB. Analysis of [U-$^{13}C_6$]glucose in human plasma using liquid chromatography/isotope ratio mass spectrometry compared with two other mass spectrometry techniques. Rapid Commun Mass Spectrom. 2009;23:3824-30.

32. Verbeke K, Ferchaud-Roucher V, Preston T, Small AC, Henckaerts L, Krempf M, Wang H, Vonk RJ, Priebe MG. Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers. Eur J Clin Nutr. 2010;64:678-84.

33. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: Lester P, editor. Methods in Enzymology Oxidants and Antioxidants Part A. Volume 299 ed. Academic Press; 1999. p. 152-78.

34. Tissot S, Normand S, Guilluy R, Pachiaudi C, Beylot M, Laville M, Cohen R, Mornex R, Riou JP. Use of a new gas chromatograph isotope ratio mass spectrometer to trace exogenous ^{13}C labelled glucose at a very low level of enrichment in man. Diabetologia. 1990;33:449-56.

35. Bradley DC, Steil GM, Bergman RN. OOPSEG: a data smoothing program for quantitation and isolation of random measurement error. Comput Methods Programs Biomed. 1995;46:67-77.

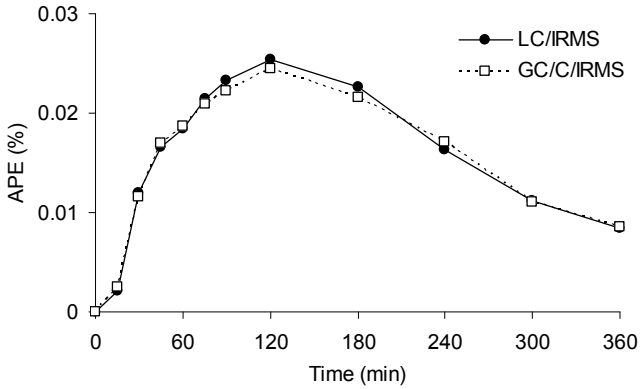
36. Schenk S, Davidson CJ, Zderic TW, Byerley LO, Coyle EF. Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am J Clin Nutr. 2003;78:742-8.

37. Clegg ME, Pratt M, Meade CM, Henry JK. The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response. Br J Nutr. 2011.

38. Patras A, Brunton N, O'Donnell C, Tiwari BK. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends Food Sci Tech. 2010;21:3-11.

39. Li W, Pickard MD, Beta T. Effect of thermal processing on antioxidant properties of purple wheat bran. Food Chem. 2007;104:1080-6.

ONLINE SUPPORTING MATERIAL



Supplemental Figure 1. *Representative graph comparing Atom Percent Excess (APE) values obtained with liquid chromatography/isotope ratio mass spectrometry (LC/IRMS) and gas chromatography/combustion (GC/C)/IRMS. Plasma from 1 participant after consumption of 132 g ^{13}C-enriched control bread with normal wheat bran (CB) was used.*

Slowly and rapidly digestible starchy foods can give a similar glycemic response due to differential tissue glucose uptake in healthy men

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ABSTRACT

BACKGROUND: Previously we observed that the consumption of pasta and bread resulted in a similar glycemic response, despite a slower intestinal influx rate of glucose from the pasta. Underlying mechanisms of this effect were not clear.

OBJECTIVE: Investigating the differences in glucose kinetics and hormonal response after consumption of products with slow and rapid *in vivo* starch digestibility, but with a similar glycemic response.

DESIGN: Ten healthy male volunteers participated in a cross-over study, consuming ^{13}C-enriched wheat bread or pasta while on a primed-continuous D-[6,6-$^{2}H_{2}$]glucose infusion. The dual isotope technique enabled calculation of glucose kinetics: rate of appearance of exogenous glucose (RaE), endogenous glucose production (EGP), and glucose clearance rate (GCR). In addition postprandial plasma concentrations of glucose, insulin, glucagon and glucose-dependent insulinotropic polypeptide (GIP) were analyzed.

RESULTS: GIP concentrations after pasta were lower than after bread consumption and strongly correlated with the RaE ($r=0.82$; $p<0.01$). The insulin response was also lower after pasta ($p<0.01$). In accordance with the low insulin response the GCR was lower after pasta, explaining the high glycemic response despite a low RaE.

CONCLUSIONS: Slower intestinal uptake of glucose from a starchy food product can result in lower postprandial insulin and GIP concentrations, but not necessarily in a lower glycemic response, due to a slower GCR. Even without being able to reduce postprandial glycemia, products with slowly digestible starch can have beneficial long term effects. Obviously these types of starchy products cannot be identified using the glycemic index and therefore another classification system seems necessary.

INTRODUCTION

Consumption of foods with slowly digestible starch compared to those with rapidly digestible starch may benefit the management of type 2 diabetes and may decrease the risk for the development of obesity, insulin resistance and type 2 diabetes (1-6). The GI, based on the postprandial glycemic response, is often used to derive information about the rate of starch digestion of carbohydrate-rich foods (7), by considering a high GI product as containing rapidly digestible starch and a low GI product as containing slowly digestible starch. However, the glycemic response is not only determined by the rate of glucose absorption from the ingested food, but also by the EGP and glucose uptake into tissues.

The use of stable isotopes enables the distinction between these processes, and studies show that the rate of appearance of glucose into the systemic circulation and the glycemic response of a starchy food product do not always correspond well. Previously, bread and pasta consumption were compared using the dual isotope technique, to obtain an insight into the *in vivo* digestive behavior of these starchy foods and how this relates to the glycemic response (8). Despite an apparent difference in RaE, being much slower for pasta, consumption of bread and pasta resulted in the same glycemic response. On the other hand, in a single-label study investigating the underlying glucose kinetics of breakfast cereals with a low and a high GI, the difference in glycemic response was not explained by a difference in glucose appearance but by a difference in glucose uptake into tissues, reflected by the GCR (9). These studies show the importance of determining glucose kinetics, as it is possible that a low GI product contains rapidly digestible starch and that a product that contains slowly digestible starch results in a relatively high glycemic response. Both may be beneficial, but via a different mechanism.

Several hormones play a role in the regulation of postprandial glucose kinetics and metabolism. The EGP is mainly regulated by the opposing actions of the hormones glucagon and insulin. Glucose uptake into tissue is greatly influenced by insulin, as it regulates the presence of the glucose transporter GLUT4 on the membrane of muscle cells and adipocytes as well as the subsequent glucose transport into the cells. Besides responding to elevated glucose concentrations in the circulation, the insulin response is greatly potentiated (up to 70%) by the incretin hormones GIP and GLP-1, released from the small intestine after an oral glycemic load (10-12). The incretins are thereby also important regulators of glucose kinetics. In a previous study using corn products we showed plasma GIP concentrations to correlate strongly with the RaE (13), relating GIP release to the rate of intestinal glucose absorption.

To answer the question why the glycemic response is not different after the consumption of bread and pasta, despite a large difference in RaE, as previously reported (8), we examined also the EGP and GCR and analyzed postprandial insulin, glucagon and GIP concentrations in plasma samples of this study.

SUBJECTS AND METHODS

Subjects

Ten healthy men [age 21 ± 0.5 y, BMI 23 ± 0.6 kg/m^2 (mean ± SEM)] were recruited. The criteria for exclusion were use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the BEBO foundation, Assen, The Netherlands. Each subject gave written informed consent for the study. This trial was registered at controlled-trials.com as ISRCTN42106325. A part of the data obtained in this trial was previously published (8).

Experimental design

The study was performed in a crossover manner, with at least 1 wk between each study day. The subjects were asked to refrain from consuming ^{13}C-enriched foods, like cane sugar, corn products and pineapple, for 3 d preceding the experiments and from alcohol consumption and strenuous exercise for 24 h before each study day. Food intake on the day before each experiment was individually standardized using a diary. A standard evening meal was provided at the commercial research facility (QPS Netherlands B.V.), where the subjects stayed overnight. In the evening a venous catheter was inserted in each forearm for blood collection and for infusion of D-[6,6-$^2H_2$] glucose (98% ^{2}H atom percent excess) (Isotec). Subjects fasted overnight, but were allowed to drink water. In the morning (t = -120), 26.7 mL D-[6,6-$^2H_2$]glucose solution (80 × 0.07 mg/kg body weight) was infused, and a continuous infusion of 0.07 mg/kg body weight D-[6,6-$^2H_2$]glucose per min was started and maintained for 8 h. Two hours after the start of the infusion (t = 0) the test meal was ingested. During the study period physical activity was limited.

Sample collection

Blood was collected into 2 mL BD Vacutainer Fluoride tubes and 3 mL BD™ P700 Blood Collection Systems containing DPP-IV inhibitor for GLP-1 preservation (BD Diagnostics). Three basal blood samples were collected (t = -60, t = -30, t = -5) and after the test meal samples were drawn every 15 min for 2 h and every 30 min for an additional 4 h. After centrifugation (1300 × g for 10 min at 4°C), plasma aliquots were stored at –20°C until analysis. Breath samples were collected by breathing through a straw into 10 mL Exetainer vials (Labco Limited). Two basal breath samples were collected (t = -30, t = -5) and after the test meal a sample was taken every 30 min until t = 360.

Test meals

The wheat-based test meals were 132 g bread and 119 g pasta (uncooked weight), prepared at TNO, Zeist, The Netherlands. Wheat bran (English Biscuit Wheat, Meneba) was added, resulting in 7% dietary fiber in the final product. Meals were ^{13}C-enriched by the addition of 12% ^{13}C-labeled wheat [*T. aestivum* var *Paragon* (1.359 Atom % ^{13}C)] cultured in a $^{13}CO_2$ enriched atmosphere, as described before14. Both test meals consisted of 50 g available carbohydrates and were consumed together

with 10 g light margarine (4 g fat), 2 slices lean ham (5 g fat, 6 g protein) and 250 mL tap water within 20 min.

The bread was prepared with 1110 g unlabeled white wheat flour [*T. aestivum* Kolibri/Ibis (ratio 70/30) (1.085 Atom % ^{13}C)], 180 g ^{13}C-labeled wheat flour, 210 g wheat bran, 975 g water, 25 g yeast and 30 g salt. After kneading, the dough was allowed to rise for 30 min, and was baked for 30 min at 240 °C. Portions of 132 g bread were stored at -20 °C until use. Pasta was freshly prepared with 739 g wheat flour (*T. durum* de Cecco), 120 g ^{13}C-labeled wheat flour, 141 g wheat bran, 400 g water and 20 g salt. Portions of 119 g were stored at 5 °C until use and cooked for 6.5 min in 2 L water before consumption.

In vitro analysis of starch fractions and determination of ^{13}C abundance of the test meals was previously described (8).

Measurement of plasma glucose, insulin, glucagon and GIP concentrations

Plasma glucose concentrations were measured on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi) using a glucose hexokinase method. The within- and between-run CV were ≤ 2 %. The ARCHITECT® insulin assay (Abbott Laboratories) was used to determine insulin concentrations in plasma. The total CV of this chemiluminescent microparticle immunoassay was ≤ 7%. Glucagon was determined using a radioimmunoassay (KGND1, Siemens). The plasma concentrations of total GIP were measured on the Luminex 100 Total System (Luminex Corporation) using Bio-Plex Pro™ Human Diabetes Panel (BioRad Laboratories).

$^{13}CO_2$ analysis in breath samples

Analysis of ^{13}C abundance in breath CO_2 was performed using GC/IRMS (Delta Plus XL; Thermo Fisher Scientific) measuring the $^{13}C/^{12}C$ ratio versus the international standard Pee Dee Belemnite ($\delta^{13}C_{PDB,}$ in ‰).

Analysis of isotopic enrichment by GC/MS and GC/C/IRMS

Analysis of isotopic enrichment by GC requires the derivatization of plasma glucose to glucose penta acetate or an alternative suitable derivative. The sample preparation is described in detail (13, 15) elsewhere. ^2H enrichment of plasma samples was measured by GC/MS as previously described (15) and $^{13}C/^{12}C$ isotope ratio was measured in plasma using GC/C/IRMS as previously described (16, 17), both with some modifications (8).

Calculation of glucose kinetics

The molar percentage enrichment of [6,6-$^2H_2$]glucose and the ^{13}C atom% were calculated as previously described (15), except that the data were not smoothed this time. The RaT (glucose from exogenous (meal) and endogenous (hepatic) sources) was calculated from total plasma glucose concentrations and ^2H-enrichment data by using the non-steady-state equation of Steele et al. (18)

as modified by De Bodo et al. (19). It was assumed that labeled and unlabeled glucose molecules showed identical behavior. The effective volume of distribution was considered to be 200 mL/kg and the pool fraction to be 0.75 (20). The systemic RaE was calculated from the RaT and ^{13}C-enrichment data, as described by Tissot et al. (20). The EGP was calculated by subtracting RaE from RaT (20). The GCR, which reflects the tissue glucose uptake, was calculated as described by Schenk et al. (9).

Incremental areas under the curve

To determine differences in glucose kinetics and in plasma glucose, insulin, glucagon and GIP concentrations, the 0–2 h iAUC was calculated using the trapezoidal rule (21). The averages of fasting measurements were used as baseline values, and areas below baseline were not included. For the iAUC calculations of RaT, RaE and GCR, the values were multiplied by body weight. The iAUC of RaE was expressed as a percentage of the administered dose of glucose equivalents (cumulative dose %). Because EGP and glucagon were suppressed after the test meals, the area beneath baseline (dAUC) was calculated, using the mirrored graphs of these parameters.

Statistics

Data are presented as means ± SEM, n=9 (n=8 for glucagon). A mixed model was used to test for time x treatment effects. If a significant interaction was found (*P*-value < 0.05), partial tests between treatments per time point were performed. A Bonferroni correction was used to correct for multiple comparisons. If necessary, data were log-transformed before analysis. The analyses were performed with the software package SAS (release 9.1; SAS Institute). For additional indices (fasting and peak values, time to peak and iAUC) differences between meals were assessed using a two-tailed paired Student's t-test. The within-subject relationship (correlation) between variables was tested by regression analysis according to the method of Bland and Altman (22). These analyses were performed by using SPSS 16.0 for Windows (SPSS Inc., Chicago). A *P* value <0.05 was considered statistically significant.

RESULTS

Characterization of test meals

Results from the *in vitro* analysis of starch fractions and determination of ^{13}C abundance of the test meals were previously reported (8).

Postprandial glucose response

Data is available from 9 subjects, because one person did not consume the entire pasta meal. No differences were found in fasting plasma glucose concentrations ($P = 0.42$), peak values ($P = 0.55$), time to peak ($P = 0.35$), or iAUC ($P = 0.22$) after the consumption of bread and pasta (**Table 1**, **Figure 1**).

Table 1. *Indices reflecting the metabolic response after ingestion of 132 g ^{13}C-enriched control bread and 119 g ^{13}C-enriched pasta (uncooked weight) in healthy men[1]*

		Fasting values	Peak values	Time to peak (min)	iAUC (0-2 h)
Glucose (mmol/L)	Bread	5.2 ± 0.1	8.0 ± 0.3	43.3 ± 3.0	140.1 ± 17.3
	Pasta	5.3 ± 0.1	7.8 ± 0.4	40.8 ± 3.3	115.0 ± 20.6
RaT (mg/(kg·min))	Bread	2.3 ± 0.1	7.0 ± 0.3	48.3 ± 7.0	44.3 ± 1.5
	Pasta	2.3 ± 0.1	5.9 ± 0.3	38.3 ± 6.7	32.8 ± 1.2*
RaE (mg/(kg·min))	Bread	0 ± 0	4.7 ± 0.1	60.0 ± 10.3	56.0 ± 2.5
	Pasta	0 ± 0	4.0 ± 0.2*	48.3 ± 10.5	38.2 ± 1.4*
EGP[2] (mg/(kg·min))	Bread	2.3 ± 0.1	1.0 ± 0.1	133.3 ± 23.1	61.8 ± 4.6
	Pasta	2.3 ± 0.1	0.6 ± 0.1*	121.7 ± 24.2	90.9 ± 9.7*
GCR (mL/(kg·min))	Bread	2.8 ± 0.1	6.9 ± 0.4	95.0 ± 6.6	18.5 ± 2.0
	Pasta	2.8 ± 0.1	5.0 ± 0.3*	93.3 ± 9.3	8.7 ± 1.4*
Insulin (μU/mL)	Bread	6.7 ± 0.9	67.3 ± 9.4	45.0 ± 5.0	3359.5 ± 472.4
	Pasta	5.6 ± 0.5	38.1 ± 3.8*	38.3 ± 3.6*	1743.4 ± 156.5*
Glucagon[2] (pmol/L)	Bread	11.4 ± 0.9	9.1 ± 0.9	60.0 ± 8.0	80.2 ± 26.4
	Pasta	11.9 ± 1.1	9.5 ± 0.8	69.4 ± 8.5	64.5 ± 12.0
GIP (pg/mL)	Bread	70.7 ± 9.4	583.1 ± 64.8	71.7 ± 8.6	36275.1 ± 4084.9
	Pasta	53.4 ± 5.7	397.5 ± 37.5*	66.7 ± 10.9	24682.1 ± 2805.6*
$^{13}CO_2$ (%dose/h)	Bread	0 ± 0	7.3 ± 0.2	216.7 ± 9.7	4.5 ± 0.2
	Pasta	0 ± 0	7.2 ± 0.2	250.0 ± 11.2*	3.3 ± 0.1*

[1]Values are means ± SEM, n=9 (n=8 for glucagon). Differences were assessed using a two-tailed paired Student's t-test.
** Significantly different from bread, P < 0.05.*
EGP, endogenous glucose production; GCR, glucose clearance rate; GIP, glucose-dependent insulinotropic polypeptide; iAUC, incremental AUC; RaE, rate of appearance of exogenous glucose; RaT, rate of appearance of total glucose
[2]Because EGP and glucagon were suppressed after the test meals, the nadir values and time to nadir are presented. Also, the area beneath baseline (dAUC) was calculated using mirrored data.

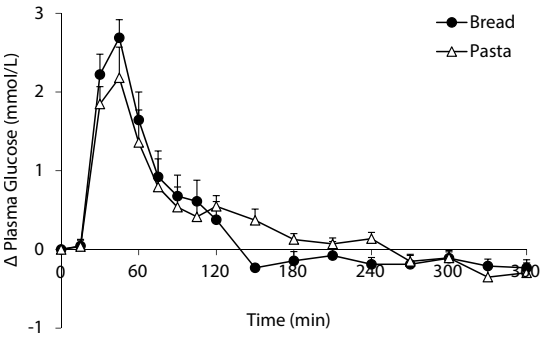

Figure 1. *Mean (± SEM) changes from baseline in plasma glucose concentrations after ingestion of 132 g ^{13}C-enriched control bread (●) and 119 g ^{13}C-enriched pasta (Δ) in healthy men (n=9). There was a significant time × treatment interaction (P = 0.0190), but no differences between treatments per time point were found after Bonferroni correction.*

Glucose kinetics

The results are summarized in Table 1. The RaE was slower after pasta consumption compared with bread intake (**Figure 2**), resulting in a lower 0-2 h iAUC ($P < 0.0001$). The EGP was more suppressed after consumption of pasta compared with bread (dAUC, $P = 0.03$). Comparing the GCR after both test meals showed that after pasta consumption glucose was cleared from the circulation at a lower rate (**Figure 3**). The 0-2 h iAUC was more than 50% lower after pasta ($P = 0.0002$).

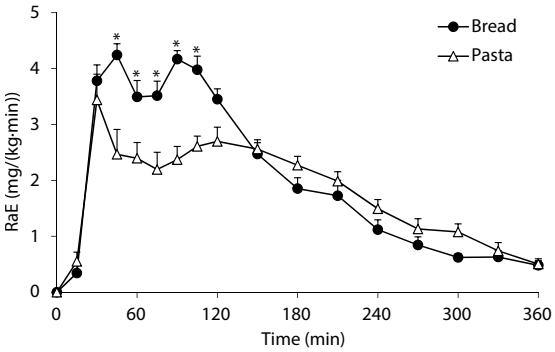

Figure 2. *Mean (± SEM) RaE after ingestion of 132 g ^{13}C-enriched control bread (●) and 119 g ^{13}C-enriched pasta (Δ) in healthy men (n=9). There was a significant time × treatment interaction (P < 0.0001). Significant differences between treatments per time point (after Bonferroni correction) are indicated (*). RaE, rate of appearance of exogenous glucose.*

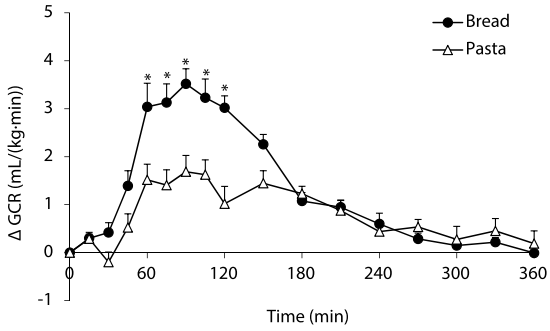

Figure 3. *Mean (± SEM) changes from baseline in GCR after ingestion of 132 g ^{13}C-enriched control bread (●) and 119 g ^{13}C-enriched pasta (Δ) in healthy men (n=9). There was a significant time × treatment interaction (P < 0.0001). Significant differences between treatments per time point (after Bonferroni correction) are indicated (*). Differences were assessed using a two-tailed paired Student's t-test. * P < 0.05. GCR, glucose clearance rate.*

Postprandial insulin, glucagon and GIP response

Insulin concentrations were lower after pasta consumption (**Figure 4**), indicated by a 48% smaller iAUC (0-2 h) compared with bread intake ($P < 0.01$) (Table 1). Glucagon concentrations after pasta were not significantly different compared with bread (data not shown). Postprandial GIP concentrations were lower after pasta compared with bread intake (**Figure 5)**, also apparent from the lower iAUC ($P = 0.002$) (Table 1).

$^{13}CO_2$ excretion in breath

For $^{13}CO_2$ excretion in breath, reflecting the rate of oxidation of the ^{13}C-labeled substrate, a significant time x treatment interaction was found ($p < 0.0001$). $^{13}CO_2$ excretion was higher after bread consumption from t = 90 until t = 180 ($P < 0.001$) and was higher after pasta consumption at t = 330 ($P = 0.003$) (data not shown). Peak values were the same after both meals ($p = 0.68$), but the time to peak was significantly later after pasta consumption ($P = 0.007$) (Table 1).

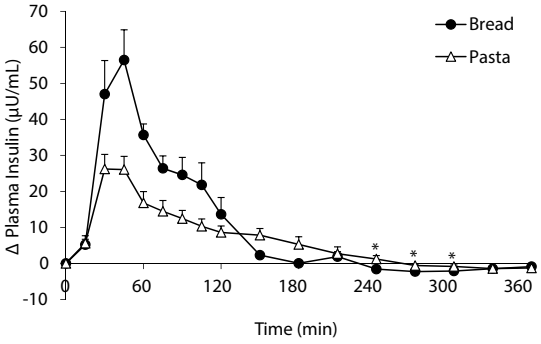

Figure 4. *Mean (± SEM) changes from baseline in plasma insulin concentrations after ingestion of 132 g ^{13}C-enriched control bread (●) and 119 g ^{13}C-enriched pasta (Δ) in healthy men (n=9). There was a significant time × treatment interaction (P < 0.0001). Significant differences between treatments per time point (after Bonferroni correction) are indicated (*).*

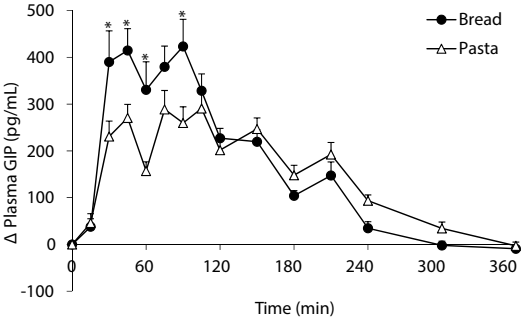

Figure 5. *Mean (± SEM) changes from baseline in plasma GIP concentrations after ingestion of 132 g ^{13}C-enriched control bread (●) and 119 g ^{13}C-enriched pasta (Δ) in healthy men (n=9). There was a significant time × treatment interaction (P < 0.0001). Significant differences between treatments per time point (after Bonferroni correction) are indicated (*). GIP, glucose-dependent insulinotropic polypeptide.*

3

Correlations

Correlations between glucose, insulin, GIP and RaE (all time points, 0-2 h; $P < 0.01$) are presented in **Table 2.** RaE and GIP showed the strongest correlation.

Table 2. *Correlation coefficients relating the glucose, insulin and GIP concentrations, and RaE after ingestion of 132 g ^{13}C-enriched control bread and 119 g ^{13}C-enriched pasta (uncooked weight) in healthy men [1]*

	Glucose	Insulin	GIP	RaE
Glucose				
Insulin	0.79			
GIP	0.48	0.60		
RaE	0.63	0.72	0.82	

[1]The within-subject relationship between variables (n=9) was tested by regression analysis according to the method of Bland and Altman (22), P < 0.01 for all reported coefficients. GIP, glucose-dependent insulinotropic polypeptide; RaE, rate of appearance of exogenous glucose

DISCUSSION

Previously we reported that bread and pasta consumption resulted in a similar glycemic response in healthy men, despite the slower glucose influx rate from the intestine (RaE) after pasta (8). These findings emphasize that the glucose response does not reflect the influx of starch-derived glucose from the food product and is the result of several metabolic processes occurring simultaneously. Because the lower RaE did not lead to a lower glycemic response, it was expected that the EGP was less suppressed and/or that the glucose uptake into tissues (GCR) was slower after pasta compared to bread, thus 'counterbalancing' the slow RaE. Therefore, the EGP and GCR were studied in detail together with the hormones influencing these processes.

The similar glycemic response after bread and pasta could not be explained by the difference in EGP, because EGP was somewhat more suppressed after pasta consumption. However, the GCR was much slower after the consumption of pasta, enabling the glucose to remain in the circulation for longer. Thus, the slower GCR after pasta consumption 'counterbalances' the slow RaE, explaining the similar glycemic response after the slowly and rapidly digestible test meal. In accordance with the lower GCR, insulin concentrations were lower after pasta than after bread consumption. However, the insulin response to pasta was much lower as would be expected from the glycemic response, as both responses often resemble each other after the consumption of starchy food products (23-25). Nevertheless, such discrepancy was also observed after intake of certain types of breads, as discussed below.

The insulin response is potentiated by the incretin hormones, and therefore the low GIP concentrations after the consumption of pasta were as expected. The low GIP response is likely explained by slower digestion of starch in pasta, which is reflected by the slow RaE. In the present study the individual patterns of RaE and GIP closely resembled each other, which is in accordance with one of our previous studies using corn products, in which GIP and RaE also showed a strong correlation (13). Duodenal perfusion studies demonstrate that GIP release dose-dependently responds to the rate of glucose delivery in the duodenum as well (26-28). Together, this means that the slow digestion of starch in pasta results in a low GIP and a subsequent low insulin response. This in turn leads to slower GCR and by that to a relatively high glycemic response.

The question then remains why some products with slowly digestible starch do result in a low glycemic response (13, 23, 29), despite of this apparent 'counterbalancing' effect. The answer could possibly be derived from two intraduodenal perfusion studies comparing glucose, insulin and incretin responses during glucose infusion ranging from 1 to 4 kcal/min (26, 28). An increase in glucose load from 1 to 2 kcal/min considerably raised the glycemic response, but there was hardly any further rise in glycemic response when 3 kcal/min (28) or 4 kcal/min (26) were administered. The absence of a further increase in glucose concentrations was explained by the substantially greater incretin and insulin responses to the higher glucose infusion rates (26, 28). GIP was suggested to account for the effect on insulin in the lower infusion range, whereas GLP-1 mainly contributed in the highest infusion range (26). The responses to the 3 – 4 kcal/min and 2 kcal/min infusion rates are

in line with our observations after bread and pasta consumption. In accordance with the infusion rate of 3 – 4 kcal/min, our bread (high RaE) resulted in a high glucose, GIP and insulin response, whereas consistent with the infusion rate of 2 kcal/min, our pasta (low RaE) resulted also in a high glucose, but a lower GIP and insulin response. Thus, it can be expected that a product with an even slower RaE than our pasta would give a response comparable to that of the 1 kcal/min infusion rate: namely a very low GIP and insulin response and a low glycemic response. We hypothesize that in this condition the reduction of GCR is not sufficient to counterbalance the very low RaE, resulting in a lower glycemic response (**Figure 6**). Further investigation is needed to determine the role of GLP-1

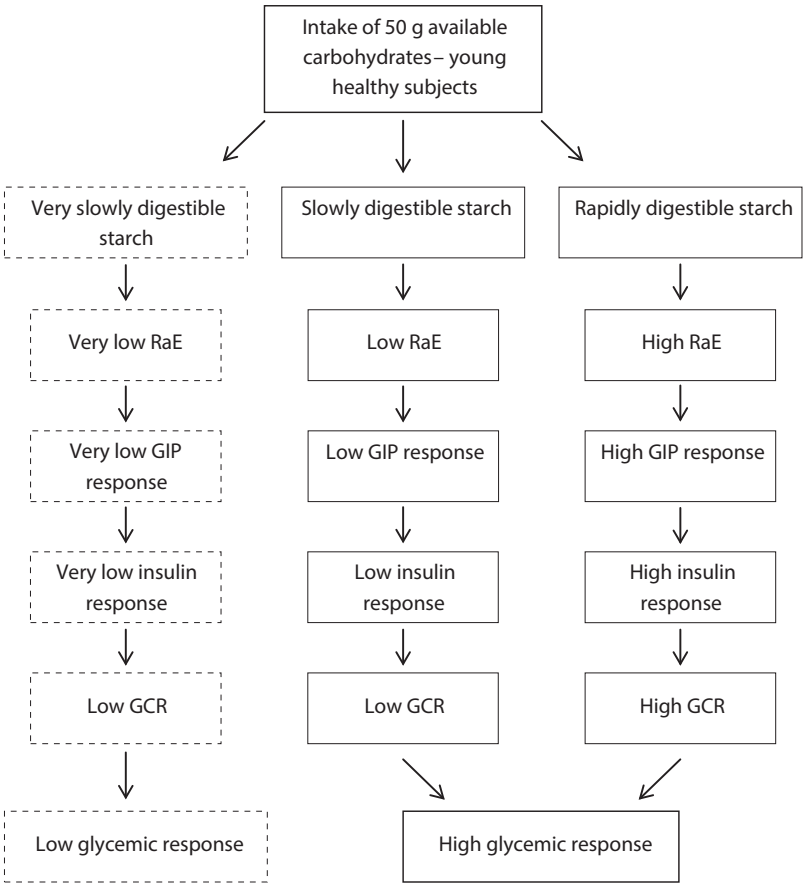

Figure 6. *Overview of results from the present study (solid text boxes), comparing a rapidly and a slowly digestible starchy food product (50 g available carbohydrates), resulting in a high glycemic response. On the left (dotted text boxes) the hypothesized situation, presumably resulting in a low glycemic response.*

and the validity of this hypothesis when there are other factors present in food that could influence GIP, insulin or GCR independently from the rate of starch digestion. For example, protein is capable of enhancing postprandial GIP and insulin secretion (30, 31) and when added to a starchy food

product likely to affect the glycemic response. It must also be noted that this study was conducted in healthy subjects, and results might be different in subjects with e.g. decreased glucose tolerance.

Our findings could shed light on the so far unexplained discrepancy between glucose and insulin response after the consumption of certain types of bread. For instance, several rye breads gave a glycemic response comparable with that of white wheat bread, whereas the insulin and incretin response were significantly lower (32-34). Glucose kinetics after consumption of these rye breads were not studied; however, based on the observed correlations between glucose influx and GIP concentrations (13, 26, 28), it is very likely that the lower GIP, GLP-1 and insulin concentrations after these rye products can be explained by a lower RaE. Indications for a slower digestibility of starch in these products were the lower hydrolysis indices compared to white wheat bread (32, 33) This was explained by a decreased accessibility of starch by degrading enzymes due to a different (microscopic) structure of rye breads (33). In addition, it can be expected that the lower insulin concentrations after rye bread result in a reduced GCR, thus showing the same counterbalancing effect as in the present study, explaining the similar glycemic response after the white wheat and rye breads. Similarly, the consumption of several differently processed wheat breads also resulted in a similar glucose, but a lower GIP (35) or insulin response (36) compared with 'conventional' breads. Here, the differences in bread preparation were suggested to have decreased the availability of starch, as indicated by the lower content of rapidly available glucose (35) or a more rigid bread structure (36).

Consumption of slowly digestible products, although not always resulting in a low glycemic response, might have several beneficial effects. Because glucose enters the circulation at a slower rate, less insulin is secreted to keep glucose concentrations beneath an acceptable limit, which would be less demanding for the pancreatic β-cells. The modest insulin response also prevents a drop in glucose concentrations beneath baseline, which is associated with increased food intake (1). The effect of a low insulin response on the inflammatory status, associated with an increased risk for the development of type 2 diabetes, was studied in a 12 wk intervention study in individuals with the metabolic syndrome. A slightly positive modulation of inflammation markers after the 'low insulin diet' was found (37), whereas the 'high insulin diet' upregulated genes related to inflammation and oxidative stress (38). With the knowledge of the present study in mind, these findings might also be explained by a slower RaE, GCR and/or oxidation rate after the 'low insulin diet', likely resulting in less oxidative stress and inflammation. Low postprandial GIP concentrations might also be favorable, as several studies have indicated that GIP might be involved in the development of obesity, due to its anabolic effects in adipose tissue, such as stimulation of fatty acid synthesis and enhancement of insulin-stimulated incorporation of fatty acids into triglycerides (39-42).

In conclusion, a high glycemic response after pasta consumption, despite slow starch digestion, can be explained by slower uptake of glucose into tissues. This is likely the result of a low insulin response, caused by the low GIP response due to slow influx of glucose from the pasta meal. Foods with slowly digestible starch, even without being able to reduce postprandial glycemia, are

preferable to those with rapidly digestible starch because of the expected beneficial long term effects. Obviously these products cannot be identified with the GI as they would fall into the high GI category. Therefore another classification system seems necessary which would be able to reflect these more subtle differences. Based on this study insulin or GIP seem to be promising candidates.

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REFERENCES

1. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 2002;287:2414-23.
2. Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care 1997;20:545-50.
3. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA 1997;277:472-7.
4. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 2002;76:274S-80S.
5. Thomas D, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. Cochrane Database Syst Rev 2009;21:Art. No.: CD006296.
6. Thomas D, Elliott EJ, Baur L. Low glycaemic index or low glycaemic load diets for overweight and obesity. Cochrane Database Syst Rev 2007;CD005105.
7. Jenkins DJA, Wolever TMS, Taylor RH. Glycemic index of foods: A physiological basis for carbohydrate exchange. Am J Clin Nutr 1981;34:362-6.
8. Eelderink C, Moerdijk-Poortvliet TC, Wang H, Schepers M, Preston T, Boer T, Vonk RJ, Schierbeek H, Priebe MG. The glycemic response does not reflect the *in vivo* starch digestibility of fiber-rich wheat products in healthy men. J Nutr 2012;142:258-63.
9. Schenk S, Davidson CJ, Zderic TW, Byerley LO, Coyle EF. Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am J Clin Nutr 2003;78:742-8.
10. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 1986;63:492-8.
11. Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J Clin Invest 1967;46:1954-62.
12. Vilsbøll T, Holst JJ. Incretins, insulin secretion and Type 2 diabetes mellitus. Diabetologia 2004;47:357-66.
13. Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Englyst KN, Holst JJ, Stellaard F, Vonk RJ. The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic polypeptide concentrations in healthy men. J Nutr 2006;136:1511-6.
14. Verbeke K, Ferchaud-Roucher V, Preston T, Small AC, Henckaerts L, Krempf M, Wang H, Vonk RJ, Priebe MG. Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers. Eur J Clin Nutr 2010;64:678-84.
15. Vonk RJ, Stellaard F, Priebe MG, Koetse HA, Hagedoorn RE, De BS, Elzinga H, Lenoir-Wijnkoop I, Antoine JM. The $^{13}C/^{2}H$-glucose test for determination of small intestinal lactase activity. Eur J Clin Invest 2001;31:226-33.
16. Vonk RJ, Hagedoorn RE, De Graaff R, Elzinga H, Tabak S, Yang YX, Stellaard F. Digestion of so-called resistant starch sources in the human small intestine. Am J Clin Nutr 2000;72:432-8.
17. Vonk RJ, Lin Y, Koetse HA, Huang CY, Zeng G, Elzinga H, Antoine JM, Stellaard F. Lactose (mal)digestion evaluated by the ^{13}C-lactose digestion test. Eur J Clin Invest 2000;30:140-6.
18. Steele R, Wall JS, De Bodo RC, Altszuler N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 1956;187:15-24.
19. De Bodo RC, Steele R, Altszuler N, Dunn A, Bishop JS. On the hormonal regulation of carbohydrate metabolism; studies with C14 glucose. Recent Prog Horm Res 1963;19:445-88.
20. Tissot S, Normand S, Guilluy R, Pachiaudi C, Beylot M, Laville M, Cohen R, Mornex R, Riou JP. Use of a new gas chromatograph isotope ratio mass spectrometer to trace exogenous ^{13}C labelled glucose at a very low level of enrichment in man. Diabetologia 1990;33:449-56.

21. Food and Agriculture Organization of the United Nations & World Health Organization. Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation. 1998.

22. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1-Correlation within subjects. BMJ 1995;310:446.

23. Björck I, Liljeberg H, Ostman E. Low glycaemic-index foods. Br J Nutr 2000;83 Suppl 1:S149-S155.

24. Holt SHA, Brand Miller JC, Petocz P. An insulin index of foods: The insulin demand generated by 1000-kJ portions of common foods. Am J Clin Nutr 1997;66:1264-76.

25. Lin MH, Wu MC, Lu S, Lin J. Glycemic index, glycemic load and insulinemic index of Chinese starchy foods. World J Gastroenterol 2010;16:4973-9.

26. Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Smout AJPM, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. Am J Physiol Endocrinol Metab 2007;293:E743-E753.

27. Schirra J, Katschinski M, Weidmann C, Schäfer T, Wank U, Arnold R, Göke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 1996;97:92-103.

28. Trahair LG, Horowitz M, Rayner CK, Gentilcore D, Lange K, Wishart JM, Jones KL. Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. J Clin Endocrinol Metab 2012;97:844-51.

29. Granfeldt Y, Björck I, Hagander B. On the importance of processing conditions, product thickness and egg addition for the glycaemic and hormonal responses to pasta: A comparison with bread made from 'pasta ingredients'. Eur J Clin Nutr 1991;45:489-99.

30. Frid AH, Nilsson M, Holst JJ, Bjorck IM. Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. Am J Clin Nutr 2005;82:69-75.

31. Gannon MC, Nuttall FQ, Neil BJ, Westphal SA. The insulin and glucose responses to meals of glucose plus various proteins in type II diabetic subjects. Metabolism 1988;37:1081-8.

32. Juntunen KS, Niskanen LK, Liukkonen KH, Poutanen KS, Holst JJ, Mykkänen HM. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. Am J Clin Nutr 2002;75:254-62.

33. Juntunen KS, Laaksonen DE, Autio K, Niskanen LK, Holst JJ, Savolainen KE, Liukkonen KH, Poutanen KS, Mykkänen HM. Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread. Am J Clin Nutr 2003;78:957-64.

34. Leinonen K, Liukkonen K, Poutanen K, Uusitupa M, Mykkänen H. Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. Eur J Clin Nutr 1999;53:262-7.

35. Bakhøj S, Flint A, Holst JJ, Tetens I. Lowel glucose-dependent insulinotropic polypeptide (GIP) response but similar glucagon-like peptide 1 (GLP-1), glycaemic, and insulinaemic response to ancient wheat compared to modern wheat depends on processing. Eur J Clin Nutr 2003;57:1254-61.

36. Rizkalla SW, Laromiguiere M, Champ M, Bruzzo F, Boillot J, Slama G. Effect of baking process on postprandial metabolic consequences: Randomized trials in normal and type 2 diabetic subjects. Eur J Clin Nutr 2007;61:175-83.

37. Kallio P, Kolehmainen M, Laaksonen DE, Pulkkinen L, Atalay M, Mykkänen H, Uusitupa M, Poutanen K, Niskanen L. Inflammation markers are modulated by responses to diets differing in postprandial insulin responses in individuals with the metabolic syndrome. Am J Clin Nutr 2008;87:1497-503.

38. Kallio P, Kolehmainen M, Laaksonen DE, Kekäläinen J, Salopuro T, Sivenius K, Pulkkinen L, Mykkänen HM, Niskanen L, Uusitupa M et al. Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome: the FUNGENUT Study. Am J Clin Nutr 2007;85:1417-27.

39. Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131-57.

40. Paschetta E, Hvalryg M, Musso G. Glucose-dependent insulinotropic polypeptide: from pathophysiology to therapeutic opportunities in obesity-associated disorders. Obes Rev 2011;12:813-28.

41. Song DH, Wolfe MM. Glucose-dependent insulinotropic polypeptide and its role in obesity. Curr Opin in Endocrinol Diabetes Obes 2007;14:46-51.

42. Yip RGC, Wolfe MM. GIP biology and fat metabolism. Life Sciences 2000;66:91-103.

The structure of wheat bread influences the postprandial metabolic response in healthy men

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ABSTRACT

Postprandial high glucose and insulin responses after starchy food consumption, associated with an increased risk of developing several metabolic diseases, could possibly be improved by altering food structure. We investigated the influence of a compact food structure; different wheat products with a similar composition were created using different processing conditions. The postprandial glucose kinetics and metabolic response to bread with a compact structure (flat bread, FB) was compared to bread with a porous structure (control bread, CB) in a randomized, crossover study with ten healthy male volunteers. Pasta (PA), with a very compact structure, was used as control. The rate of appearance of exogenous glucose (RaE), endogenous glucose production, and glucose clearance rate (GCR) was calculated using stable isotopes. Furthermore, postprandial plasma concentrations of glucose, insulin, several intestinal hormones and bile acids were analyzed.

The structure of FB was considerably more compact compared to CB, as confirmed by microscopy, XRT analysis (porosity) and density measurements. Consumption of FB resulted in lower peak glucose, insulin and glucose-dependent insulinotropic polypeptide (ns) responses and a slower initial RaE compared to CB. These variables were similar to the PA response, except for RaE which remained slower over a longer period after PA consumption. Interestingly, the GCR after FB was higher than expected based on the insulin response, indicating increased insulin sensitivity or insulin-independent glucose disposal. These results demonstrate that the structure of wheat bread can influence the postprandial metabolic response, with a more compact structure being more beneficial for health. Bread-making technology should be further explored to create healthier products.

INTRODUCTION

Frequent consumption of starchy foods that result in a high postprandial blood glucose response may contribute to an increased risk of developing type 2 diabetes (1). In addition, high postprandial insulin concentrations could play a role in developing obesity (2) and insulin resistance (3). Therefore, efforts to modulate the postprandial glucose and insulin responses after consumption of starchy foods are highly relevant.

The glycemic response after starchy foods is determined by three glucose fluxes: glucose which is derived from digested starch entering the circulation (RaE: rate of appearance of exogenous glucose), glucose produced in the liver (EGP: endogenous glucose production) and glucose uptake into tissues (GCR: glucose clearance rate). The postprandial glucose-induced rise in insulin concentrations, facilitating glucose uptake into tissue, can be potentiated by up to 70% by the incretin hormones; glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (4). They are released postprandial from intestinal K- and L-cells, respectively, and have several other physiological functions. GLP-1 is involved in the regulation of gastric emptying (5) and satiety (6) and, in rodents, found to preserve β-cell function (7). GIP may be implicated, at least in animals, with increased nutrient uptake into adipocytes and fat accumulation (4, 8), and inhibition of GIP signaling in mice prevented obesity (9). It has been shown that GIP plasma concentrations are closely correlated with the RaE (10, 11). Therefore, slow starch digestion may also exert beneficial effects by reducing GIP release. The relationship of GLP-1 and RaE is weaker (10) and in contrast to GIP, GLP-1 would need to be elevated to exert its beneficial effects.

One way to influence postprandial glucose kinetics is by applying different food processing techniques and by consequence changing food structure. A slower RaE, reflecting decreased starch digestibility, was observed after ingestion of breakfast biscuits, which were produced from the same ingredients, but underwent different processing conditions than breakfast cereals (12). Extrusion rendered starch granules in breakfast cereals more accessible to starch digestion, whereas the integrity of starch granules was preserved by preventing complete gelatinization of starch by the biscuit making process (heating with low water content). Previously, we compared postprandial glucose kinetics of fiber-rich fresh pasta and wheat bread which were composed of similar ingredients, but were processed differently (11). The RaE was slower and the insulin response lower after pasta than after bread, but unexpectedly, total blood glucose did not differ. The same discrepancy between the glucose and insulin response was observed in several studies comparing different types of wheat (13) and rye breads (14, 15). A common characteristic of the products exerting this effect was their relatively compact food structure - likely resulting in slow starch digestion - which was in agreement with the observation for pasta in our previous study (11). After consumption of starchy products with a compact food structure, the food particles leaving the stomach can still be relatively compact, and accordingly the accessibility to α-amylase is reduced (16), resulting in a slower RaE. As a consequence GIP is lower and results in a lower insulin response (11). This causes a slower GCR and therefore total blood glucose is not necessarily decreased. Products with slowly digestible

starch could thus exert beneficial effects by reducing insulin and GIP concentrations even without lowering total postprandial blood glucose.

Bread is widely consumed worldwide, but many types result in undesirable high glucose and insulin responses (17), whereas the glucose kinetics and GIP response are mostly unknown. We aimed to produce bread with a compact structure, flat bread, by changing processing conditions, and compared this to a porous control bread and compact wheat pasta. All high-fiber products were manufactured from the same ingredients, except for the addition of yeast to the control bread, thus only differed in structure. We investigated whether consumption of this flat bread could result in a more beneficial postprandial 'pasta-type' response compared to the control bread. For characterizing the metabolic response, we measured glucose kinetics, insulin, GIP and GLP-1, various bile acids (BA) and cholecystokinin (CCK).

SUBJECTS AND METHODS

Subjects

Ten healthy men [age 24 ± 0.6 y, BMI 22 ± 0.2 kg/m^2 (mean ± SEM)] were recruited (Sept-Oct 2011). The main criteria for exclusion were use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the 'Beoordeling Ethiek Biomedisch Onderzoek' foundation, Assen, The Netherlands. Each subject gave written informed consent for the study. This trial was registered at trialregister.nl as NTR3020.

Test meals

Three wheat-based test products were prepared (TNO, Zeist, The Netherlands) that had a similar composition, but differed in food structure due to different processing conditions: control bread (CB), flat bread (FB), and pasta (PA).

By conventional roller-milling, refined white flour and wheat bran were obtained from unlabeled (1.085 Atom % ^{13}C) wheat [T. *aestivum* var *Capo*, grown in Austria]. The products were ^{13}C-enriched by using 12% whole meal flour from ^{13}C-labeled wheat [*T. aestivum* var *Paragon* (1.359 Atom % ^{13}C)] cultured in a $^{13}CO_2$ enriched atmosphere.

The control bread (CB) was prepared with 1446 g unlabeled white wheat flour, 240 g ^{13}C-labeled whole meal wheat flour, 314 g wheat bran, 1300 g water, 33.4 g yeast, 36 g salt, 3 g malt and 70 ppm ascorbic acid. After kneading, the dough was left to rise for 30 min, moulded, and left to rise for 60 minutes. Subsequently, the bread was baked for 30 min at 240 °C.

Flat bread (FB) was prepared with 1450 g unlabeled white wheat flour, 240 g ^{13}C-labeled whole meal wheat flour, 310 g wheat bran, 1300 g water, 36 g salt, and 3 g malt. After kneading, the dough was left to rest for 30 min, sheeted to 1.5 mm thickness and disks with a diameter of 14 cm were cut. The thin dough pieces were baked on a hot stone plate in an oven at 350 °C for 30 s at each side to provide flat breads with a brown colored dry crust and a soft moist crumb inside. Bread portions

were stored at -20 °C until use.

Pasta (PA) was prepared with 763 g unlabeled white wheat flour, 120 g ^{13}C-labeled whole meal wheat flour, 117 g wheat bran, 390 g water and 20 g salt. Dough was mixed in a z-blade mixer and spaghetti was prepared using a sheeting method, creating an elastic dough sheet after multiple rolling and folding steps. This dough sheet was cut in spaghetti strings which were about 2*2 mm and 25 cm long. Portions of pasta dough were stored at -20 °C until use and cooked for 3 min in 2 L water before consumption.

All test meals provided 50 g available carbohydrates; portion sizes were 138 g for CB, 119 g for FB, and 127 g for PA (uncooked weight). The breads and pasta were consumed together with 10 g margarine light (4 g fat), 2 slices lean ham (5 g fat, 6 g protein) and 250 mL tap water within 20 min.

Experimental design

The total registered study, addressing two different research questions, consisted of 4 test products. To increase clarity and be able to focus on the results of one of these questions, three meals are described in this paper and a part is described elsewhere (18). The study was performed in a randomized, crossover manner, with at least 1 wk between each study day. The subjects were asked to refrain from consuming ^{13}C-enriched foods, such as cane sugar, corn products and pineapple, for 3 d preceding the experiments and from alcohol consumption and strenuous exercise for 24 h before each study day. Food intake on the day before each experiment was individually standardized using a diary.

A standard evening meal was provided at the commercial research facility (QPS Netherlands B.V.), where the men stayed overnight. In the evening, a venous catheter was inserted in each forearm for blood collection and for infusion of D-[6,6-$^2H_2$]glucose (98% ^2H atom percent excess) (Isotec). Subjects fasted overnight, but were allowed to drink water. In the morning (t = -122 min), 26.7 mL D-[6,6-$^2H_2$]glucose solution (80 × 0.07 mg/kg body weight) was infused, and a continuous infusion of 0.07 mg/kg body weight D-[6,6-$^2H_2$]glucose per min was started (t = -120 min) and maintained for 8 h (until t = 360 min). Two hours after the start of the infusion, the test meal was ingested (t = 0 min). Water (150 mL) was provided hourly, starting at t = 120 min. Physical activity was limited during the day.

Test meal characterization

Starch, dietary fiber and moisture contents were determined [Starch, enzymatic method (W55110); Total Dietary Fiber (AOAC), Gravimetric method (W5586); Moisture 102 °C, Gravimetric method (W5549)] at Eurofins Analytico Food, The Netherlands. To quantify starch fractions (G_T, G_{RA}, G_{SA}, G_{TA} and RS) of the test meals *in vitro*, an adapted version of the Englyst method (19) was used (20).

Bread density (g/mL) was calculated by the bread weight (g) divided by volume (mL), which was determined by rapeseed displacement in duplicate. The density of cooked PA was measured in 10-fold (10 replicates of 1 pasta string) by paraffin oil displacement.

Stereomicroscopy was performed without sample preparation using an Olympus SZX-9 microscope connected with a DP-50 digital camera (settings: Red 1.22; Green 1.22 and Blue 1.22). Light microscopy was performed with an Olympus BH-2 light microscope and the same digital camera type and settings. Sections of 10 µm thickness were cut in a cryostat-microtome at -26°C and applied on gelatin/glycerol coated slides. Sections were stained for 1 minute with Lugol (KI-I2) to color the starch (blue) and for 10 minutes with 0.05 % Ponceau 2R solution in 50% glycerol in water to color the protein (red). The excess of stain was rinsed with a minimum of water and the sections were covered with glycerol.

To determine test meal porosity, samples were scanned using a desktop X-ray microtomography (XRT) system (Model 1172, SkyScan, Aartselaar, Belgium) consisting of an X-ray tube, an X-ray detector and a CCD camera. The X-ray tube was operated at a voltage of 40 kV/250 µA to obtain optimum contrast between void (air cells) and matter (cell walls) according to a modified method. A 12-bit cooled CCD camera (512 x 1024 pixels) was used to collect the X-ray data. Bread samples were sealed in small bags and rotated by a total of 180° during the scanning process with a pixel size of 24.31 µm to obtain optimum resolution, which gave a total scanning time of 18 min. Pasta samples were cooked in boiling water as described above and then sealed in microcentrifuge tubes with water to avoid drying out during the scanning period. Pasta samples were rotated by a total of 180° with a pixel size of 4.86 µm and a total scanning time of 30 min. The initial X-ray radiographs or raw images were obtained at every 0.7° of rotation. Samples were scanned in triplicate. After scanning, radiographs were loaded into NRecon reconstruction software (v. 1.6.6). The software combines the images graphically into a 3-D object from which 2-D cross sectional images can be taken. Before the reconstruction, the CS rotation feature was used to rotate the sample cross sections, making them parallel to the view window. Beam hardening correction was set to 40% in order to reduce the number of artefacts. Cell walls of the solid matrix appear grey, whereas air cells appear black. The reconstructed 2-D slices were then loaded into CTAn software (v. 1.12, Skyscan, Belgium) to obtain the parameters of porosity, air cell wall thickness, and air cell diameter.

Sample collection

Blood was collected into 2 mL fluoride tubes (NaF), 3 mL EDTA tubes [+ 30 µL DPP-4 inhibitor (Millipore)], and 3 mL Lithium/Heparin tubes (BD Diagnostics). Three basal blood samples were collected (t = -60, t = -30, t = -15 min) and postprandial samples were drawn every 15 min for 2 h, every 30 min for an additional 3 h, and once after 6 h. To obtain heparin plasma, blood was collected at t = -60, t = -15 min and then every 30 min for the first 3 hours, and hourly for the last 3 hours. After centrifugation (1300 × g for 10 min at 4°C), plasma aliquots were stored at –20°C (NaF and EDTA plasma) or –80°C (heparin plasma) until analysis.

Breath samples were collected by breathing through a straw into 10 mL Exetainer vials (Labco Limited). Two basal breath samples were collected (t = -30, t = -5 min) and after the test meal a sample was taken every 30 min until t = 360 min.

Subjects were asked to rate their feeling of hunger using a visual analogue scale (VAS) 15 min before and hourly after the test meal. At the same time points, their feeling and extent of discomfort (abdominal pain, flatulence, other complaints) were recorded (0 = no complaints, 3 = severe complaints). The liking of the test meal was rated using a VAS (0 = not tasty, 100 = very tasty) half an hour after the start of test meal consumption (t = 30 min).

Measurement of plasma glucose, insulin, glucagon, incretin and CCK concentrations

Plasma glucose concentrations were measured on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi) using a glucose hexokinase method. The within- and between-run CV were ≤ 2 %. The ARCHITECT® insulin assay (Abbott Laboratories) was used to determine insulin concentrations in plasma. The total CV of this chemiluminescent microparticle immunoassay was ≤ 7%. The glucagon assay was directed against the C-terminal of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin (21).

Total GIP was measured using the C-terminally directed antiserum (code no. 80867) (22), which reacts fully with intact GIP (1-42) and the N-terminally truncated metabolite GIP (3–42). Total GLP-1 concentrations were determined as previously described (23), using a radioimmunoassay (antiserum no. 89390) specific for the C-terminal of the GLP-1 molecule and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. The glucagon and incretin assays have detection limits of < 2 pmol/L and an intra-assay CV of approximately 6%. CCK was measured using antiserum no. 92128, which binds the bioactive forms of CCK with equal potency without cross-reactivity with any gastrin (24). The detection limit of the CCK assay is 0.1 pmol/L, and the intra-assay CV approximately 5%.

Measurement of plasma bile acids

Fasting and postprandial concentrations of 15 individual BAs were determined using LC/MS; the primary BAs [cholic acid (CA) and chenodeoxycholic acid (CDCA)], the secondary BAs [deoxycholic acid (DCA), ursodeoxycholic acid (UDCA) and lithocholic acid (LCA)], as well as their glycine (G, glyco-) and taurine (T, tauro-) conjugates. Concentrations of 7 of the BAs were below the lower limit of quantitation (LOQ) of 0.05 µM. The intra- and inter-assay CV ranged from 1.6% to 11.3% and 4.4% to 13.3%, respectively.

For sample preparation, 250 µl of internal standard solution was mixed with 25 µl plasma, and centrifuged at 15.900 x g for 10 min. The supernatant was transferred into a new vial, evaporated under nitrogen at 40 °C, and reconstituted in 100 µl of 50% methanol. The solution was filtered with a 0.2 µm centrifugal filter at 2000 x g for 10 min. After this step, the samples (injection volume 10 µl) were ready for analysis, using two different LC-MS systems.

Analysis of isotopic enrichment in breath and plasma samples

Analysis of ^{13}C abundance in breath CO_2 was performed using GC/IRMS (Delta Plus XL; Thermo Fisher

Scientific) measuring the $^{13}C/^{12}C$ ratio versus the international standard Pee Dee Belemnite ($\delta^{13}C_{PDB,}$ in ‰).

Plasma sample preparation required for analysis of isotopic enrichment by GC (derivatization) is described in detail elsewhere (10, 25). ^{2}H enrichment was measured by GC/MS as previously described (25) and $^{13}C/^{12}C$ isotope ratio was measured using GC/C/IRMS as previously described (26), both with some modifications (27).

Calculation of glucose kinetics

The molar percentage enrichment of [6,6-$^{2}H_{2}$]glucose and the ^{13}C atom% were calculated as previously described (25), except that data were not smoothed this time. The rate of appearance of total glucose [RaT, glucose from exogenous (meal) and endogenous (hepatic) sources] was calculated from total plasma glucose concentrations and ^{2}H-enrichment data by using the non-steady-state equation of Steele et al. (28) as modified by De Bodo et al. (29) It was assumed that labeled and unlabeled glucose molecules showed identical metabolic behavior. The effective volume of distribution was assumed to be 200 mL/kg and the pool fraction to be 0.75 (30). The systemic RaE was calculated from the RaT and ^{13}C-enrichment data, as described by Tissot et al. (30). The EGP was calculated by subtracting RaE from RaT (30). The GCR, which reflects the tissue glucose uptake, was calculated as described by Schenk et al. (31).

Incremental areas under the curve

To determine differences in glucose kinetics and plasma glucose, insulin, glucagon, incretin, CCK and bile acid concentrations, the 0–2 and 0-6 h iAUC were calculated using the trapezoidal rule (32). The averages of fasting measurements were used as baseline values, and areas below baseline were not included. For the iAUC calculations of RaT, RaE and GCR, the values were multiplied by body weight. The iAUC of RaE was expressed as a percentage of the administered dose of glucose equivalents (cumulative dose %). Because EGP and unconjugated BA were suppressed after the test meals, the area beneath baseline (dAUC) was calculated.

Insulin sensitivity

Post hoc analyses of possible differences in insulin sensitivity were performed according to a previously reported adapted method (33, 34) using GCR and insulin data. The time periods 0-2 and 0-6 h, but also the time period 0-3 h were analyzed; the latter because this time period included the postprandial insulin response until it returned back to baseline.

Statistics

Data are presented as means ± SEM (n=10), unless indicated otherwise. Analyses were based on the total registered study, so including data from 4 test meals (see experimental design). For parameters that result in individual points of a time curve, we fitted a model that accounts for the

differences in the means of the 40 subject/occasion combinations, the main effects of treatment and time and the time x treatment interaction. Residual plots based on such a model were used to assess approximate normality and homogeneity of variances. Parameters that were not compatible with these assumptions were transformed before definitive analysis. We performed F tests on time × treatment interaction effects to assess whether the test meals gave rise to curves of a different shape. If the test resulted in a *P* value < 0.05, we tested differences between the meals for each time point. If there were no statistically significant differences in shapes, we tested overall differences between the meals based on the above model without the time x treatment interaction.

To assess summary measures such as iAUC, we fitted a model accounting for the differences in the means of the 10 subjects and the four occasions, and the main effects of treatment. Transformed data were used whenever residual plots showed incompatibility with normality and homogeneity assumptions. We performed an F test to see whether there were differences between the meals. If this test resulted in a *P* value < 0.05, pairwise comparisons among the meals were conducted. A Benjamini-Hochberg correction was applied on P values from all partial tests to correct for multiple comparisons, resulting in a set of differences in which at most 5% were falsely selected (*P* < 0.0052 for partial tests was considered significant). The analyses were performed with the software package GenStat, release 13.

The within-subject relationship (correlation) between variables was tested by regression analysis according to the method of Bland and Altman (35). Test meal differences (e.g. density, porosity) were assessed using a Student's t test; a *P* value < 0.05 was considered significant. These analyses were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago).

RESULTS

Characterization of test meals

Results from *in vitro* quantification of starch fractions suggested that CB and FB were most similar, whereas the PA contained approximately 10% less G_{RA} and 10% more G_{SA} (**Table 1**).

Table 1. *In vitro analysis of starch fractions in the test meals*[1,2]

Starch fraction	Test meal/portion		
	CB	FB	PA
	Total	Total	Total
		%	
G_T	100	100	100
G_{RA}	78.8	77.9	69.4
G_{SA}	11.8	9.1	20.3
G_{TA}	90.6	87.0	89.7
RS	8.4	11.7	9.3

[1]Analyses were performed in duplicate.

[2]CB, control bread; FB, flat bread; G_{RA}, rapidly available glucose (20 min); G_{SA}, slowly available glucose (20-120 min); G_T, total glucose (indicated as 100%); G_{TA}, total available glucose (120 min); PA, pasta; RS, resistant starch.

The products (CB vs FB vs PA (cooked)) varied in amount of starch (36.2, 42.1 and 20.1%), dietary fiber (7.6, 7.5 and 3.5%) and moisture content (38.6, 32.6 and 69.5%). Based on the portion size (50g available carbohydrate) the products contained 10.5, 8.9 and 8.7g dietary fiber, respectively.

Product density varied substantially between the meals (**Figure 1** A). CB had a density of 0.29 g/mL ± 0.01 compared to 0.47 g/mL ± 0.00 for FB and 1.15 g/mL ± 0.01 for PA (cooked). The difference in density was clearly visible in the product structure (Figure 1 B). Where CB had large air cells and thin cell walls due to yeast leavening, FB had a more compact structure with fewer air cells in absence of yeast leavening. Only small air cells were visible in FB originating from air incorporation during the mixing process, as well as a larger cavity in the middle of the product as a result of puffing of the dough during baking. PA had a compact structure without any air cells. This was reflected by measures of porosity using XRT (**Supplemental Table 1**). Porosity was highest for CB (83 %). FB, which had a more closed structure, had a porosity of 47%. The 3% porosity obtained for PA was mainly related to the structural disintegrates (e.g. internal cracks) coming from the added bran (**Figure 2**). Average cell wall thickness of CB was significantly smaller than of FB and the average air cell diameter was larger in CB due to the processing differences described above.

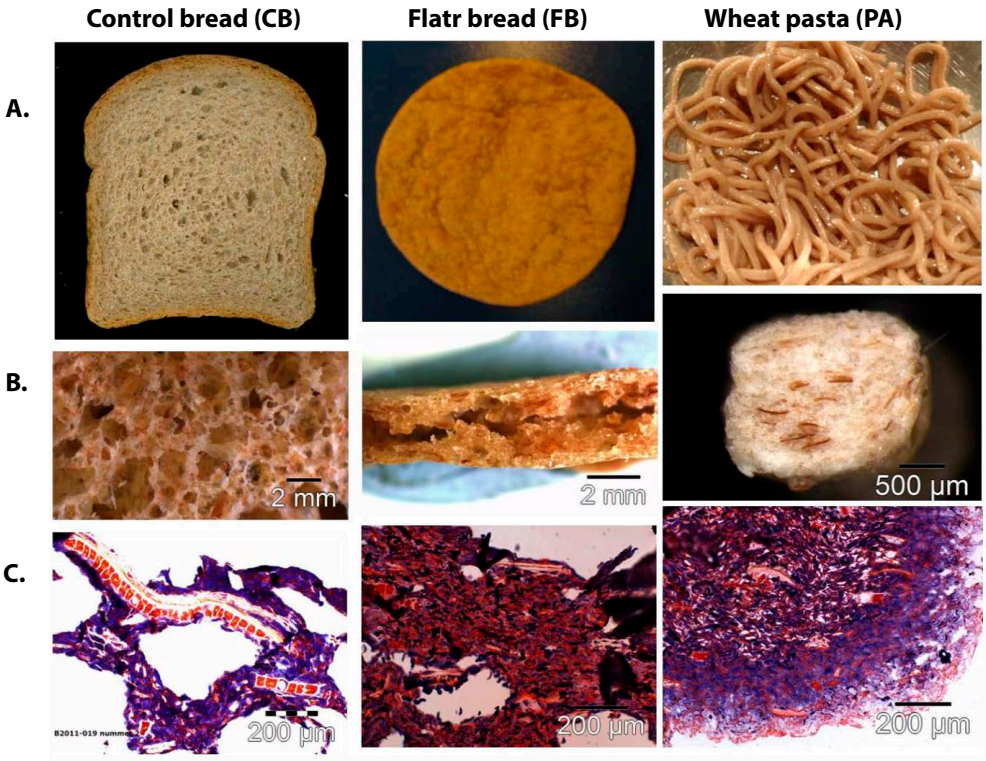

Figure 1. *Pictures of ^{13}C-enriched control bread (CB), flat bread (FB) and pasta (PA): A, overview; B, stereo microscopy; C, light microscopy with starch (lugol; blue) and protein (Ponceau 2R; red) staining.*

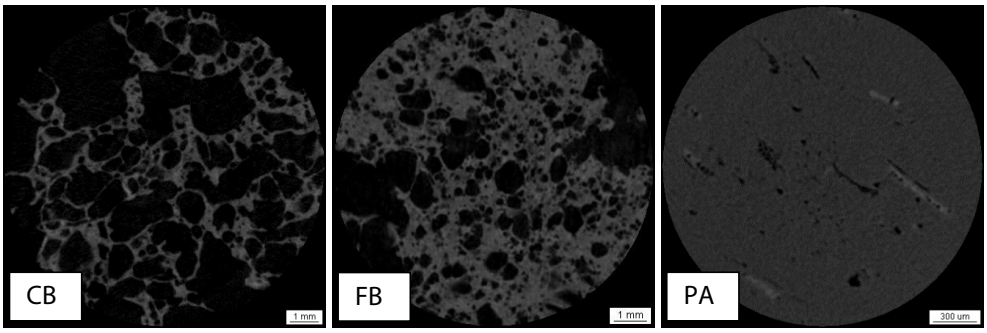

Figure 2. *Representative 2D XRT images of control bread (CB), flat bread (FB) and cooked pasta (PA).*

The porosity and average air cell diameter obtained from image analysis are closely related (r = 1.00) and inversely related to the product density as determined by physical measurement (r = -0.97 and -0.95 respectively).

The products' microstructure (Figure 1 C) showed that FB appeared to have a protein continuous phase (protein stained red) in which the starch granules are dispersed, whereas the microstructure of PA and even more of CB appeared to be dominated by swollen starch granules (starch stained blue-purple), with patches of protein. This may be explained by the very short baking process of FB, resulting in less time for complete swelling and gelatinization of the starch. For PA, a clear gradient from the center to the surface of the structure can be observed. In the center (left top corner) the starch granules were dark and compact, whereas more and more swelling of the granules was observed towards the outside, with lighter color of the granules and some colored areas in between the starch granules due to amylose leakage.

Postprandial glucose and insulin response

Postprandial glucose concentrations did not differ after the consumption of CB, FB and PA based on comparisons (all meals) of iAUC and time points (**Figure 3** A**, Table 2**). However, significant differences in glucose peak value between CB-FB (8.3 ± 0.3 vs 7.5 ± 0.2, $P < 0.002$) and CB-PA (8.3 ± 0.3 vs 7.6 ± 0.3, $P < 0.003$) were found (Table 2).

The insulin response was lower after PA consumption compared to CB at several time points (t = 45, 60, 105 min, $P < 0.005$) (Figure 3 B), which resulted in a smaller 0-6 h iAUC compared with CB consumption ($P < 0.005$, Table 2). FB and PA showed similar low insulin responses, which were lower compared to CB based on the 0-2 h iAUC ($P < 0.005$). A lower average peak value was observed after FB and PA consumption compared to CB ($P < 0.005$) as well. At t = 210 min ($P < 0.005$), insulin was higher after PA compared to CB and FB.

Table 2 *Indices reflecting the metabolic response after ingestion of 138 g ^{13}C-enriched control bread, 119 g ^{13}C-enriched flat bread and 127 g ^{13}C-enriched pasta (uncooked weight) in healthy men*[1]

	Basal concentrations	Peak concentrations	Time to peak (min)	iAUC (0-2 h)	iAUC (0-6 h)
Glucose (mmol/L)					
CB	5.1 ± 0.1	8.3 ± 0.3	40.5 ± 3.2	163.2 ± 20.5	197.0 ± 23.4
FB	5.0 ± 0.1	7.5 ± 0.2*	36.8 ± 2.4	130.1 ± 15.6	169.5 ± 17.0
PA	5.0 ± 0.1	7.6 ± 0.3*	34.5 ± 2.3	134.5 ± 22.6	191.1 ± 30.9
Insulin (µU/mL)					
CB	4.9 ± 0.5	69.9 ± 10.5	43.5 ± 3.5	3290.9 ± 528.8	4193.0 ± 936.6
FB	4.9 ± 0.5	44.5 ± 5.3*	43.5 ± 3.5	2168.9 ± 238.8*	2714.4 ± 329.0
PA	4.6 ± 0.6	36.0 ± 5.6*	37.5 ± 4.0	1689.0 ± 186.3*	2334.3 ± 248.3*
Glucagon (pmol/L)					
CB	8.3 ± 1.0	15.8 ± 1.7	175.5 ± 33.4	272.9 ± 84.6	980.5 ± 163.4
FB	8.9 ± 0.8	13.7 ± 1.4	101.3 ± 31.9	226.5 ± 52.1	587.2 ± 143.3
PA	9.6 ± 1.1	14.6 ± 1.1	147.0 ± 31.4	139.3 ± 36.9	442.3 ± 127.4
GIP (pmol/L)					
CB	9.2 ± 1.8	84.9 ± 10.5	93.0 ± 13.6	4904.6 ± 426.7	9089.2 ± 1137.8
FB	10.6 ± 2.1	68.4 ± 6.1	84.0 ± 13.5	3882.3 ± 296.0	7471.2 ± 615.3
PA	10.9 ± 0.9	63.8 ± 4.6	90.0 ± 14.8	3796.5 ± 396.7	7746.5 ± 837.9
GLP-1 (pmol/L)					
CB	15.5 ± 1.4	30.9 ± 2.1	86.3 ± 15.7	949.9 ± 95.0	2019.9 ± 183.0
FB	16.9 ± 1.7	30.7 ± 3.6	82.5 ± 14.2	765.9 ± 111.7	1489.7 ± 219.9
PA	17.1 ± 1.5	30.5 ± 3.2	121.5 ± 21.7	686.5 ± 94.5	1747.6 ± 256.3
RaT (mg/kg·min)					
CB	2.0 ± 0.1	6.5 ± 0.3	57.0 ± 14.5	39.1 ± 1.4	88.1 ± 1.6
FB	2.0 ± 0.0	5.7 ± 0.3	55.5 ± 13.0	37.0 ± 1.6	87.9 ± 2.4
PA	2.0 ± 0.1	6.0 ± 0.5	34.5 ± 3.2	33.7 ± 2.1*	84.1 ± 1.8
RaE (mg/kg·min)					
CB	0 ± 0	4.9 ± 0.2	66.0 ± 14.9	49.7 ± 1.6	95.9 ± 4.7
FB	0 ± 0	4.0 ± 0.2*	61.5 ± 13.7	43.5 ± 2.2	91.0 ± 3.3
PA	0 ± 0	3.9 ± 0.3*	48.0 ± 10.9	37.5 ± 2.9*	88.6 ± 2.6

EGP (mg/kg·min)[2]					
CB	2.0 ± 0.1	0.9 ± 0.2	111.0 ± 12.1	43.4 ± 9.3	157.8 ± 26.7
FB	2.0 ± 0.0	1.1 ± 0.2	103.5 ± 22.4	34.7 ± 8.7	133.6 ± 19.8
PA	2.0 ± 0.1	0.9 ± 0.1	100.5 ± 18.7	42.0 ± 11.1	171.6 ± 24.8
GCR (mL/kg·min)					
CB	2.5 ± 0.1	6.4 ± 0.2	100.5 ± 12.5	15.6 ± 1.5	32.7 ± 1.7
FB	2.5 ± 0.1	6.2 ± 0.3	99.0 ± 11.7	15.0 ± 1.7	33.4 ± 2.1
PA	2.5 ± 0.1	5.7 ± 0.4	111.0 ± 17.2	11.8 ± 2.0	29.1 ± 2.0
$^{13}CO_2$ (%dose/h)					
CB	0 ± 0	7.7 ± 0.2	228.0 ± 9.2	4.3 ± 0.3	31.7 ± 0.7
FB	0 ± 0	7.4 ± 0.2	243.0 ± 10.4	3.6 ± 0.3*	29.2 ± 1.0*
PA	0 ± 0	7.6 ± 0.2	267.0 ± 14.5	3.5 ± 0.3*	29.6 ± 0.8
CCK (pmol/L)					
CB	0.5 ± 0.1	2.6 ± 0.3	115.5 ± 10.5	142.4 ± 5.7	295.0 ± 26.4
FB	0.7 ± 0.1	2.5 ± 0.2	126.0 ± 10.8	122.3 ± 14.6	242.9 ± 30.8
PA	0.6 ± 0.1	2.1 ± 0.2	103.5 ± 15.2	104.1 ± 12.8*	231.9 ± 28.9*
Total BA (µmol/L)					
CB	2.1 ± 0.4	4.3 ± 0.7	61.5 ± 19.7	93.0 ± 23.3	171.2 ± 40.9
FB	2.4 ± 0.7	5.4 ± 1.1	64.5 ± 24.4	86.0 ± 19.2	162.0 ± 40.7
PA	2.0 ± 0.5	4.1 ± 0.9	87.0 ± 30.5	80.8 ± 19.9	147.4 ± 46.1
Conjugated BA (µmol/L)					
CB	1.2 ± 0.3	3.6 ± 0.6	63.0 ± 19.3	110.8 ± 21.3	216.2 ± 43.4
FB	1.4 ± 0.5	4.5 ± 0.9	90.0 ± 25.8	100.1 ± 16.9	213.5 ± 34.9
PA	1.3 ± 0.3	3.1 ± 0.5	85.5 ± 30.6	67.3 ± 18.0	133.8 ± 46.1
Unconjugated BA (µmol/L)[2]					
CB	0.9 ± 0.3	0.2 ± 0.1	211.5 ± 38.0	47.7 ± 26.9	1 85.2 ± 96.9
FB	1.0 ± 0.3	0.2 ± 0.1	271.5 ± 32.7	46.3 ± 18.1	220.1 ± 80.2
PA	0.6 ± 0.2	0.3 ± 0.2	282.0 ± 36.7	8.3 ± 3.2	49.0 ± 15.9

*[1]Values are means ± SEM, n=10. * Significantly different from control bread. BA, bile acid; CB, control bread; CCK, cholecystokinin, EGP, endogenous glucose production; FB, flat bread; GCR, glucose clearance rate; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; iAUC, incremental area under the curve; PA, pasta; RaE, rate of appearance of exogenous glucose; RaT, rate of appearance of total glucose.*

[2] Because EGP and unconjugated BA were suppressed after the test meals, the nadir concentrations and time to nadir are presented. Also, the area beneath baseline (dAUC) was calculated using mirrored data.

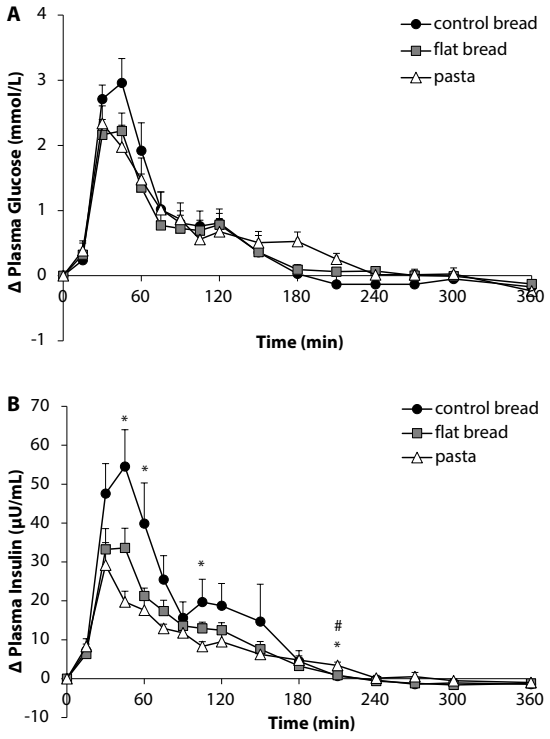

Figure 3. *Mean (± SEM) changes from baseline in (A) plasma glucose concentrations and (B) plasma insulin concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). There was no significant time × treatment interaction for glucose (P = 0.1773), but there was for insulin (P < 0.0001). *Significantly different between CB and PA per time point (after Benjamini Hochberg correction). #Significantly different between PA and FB per time point (after Benjamini Hochberg correction).*

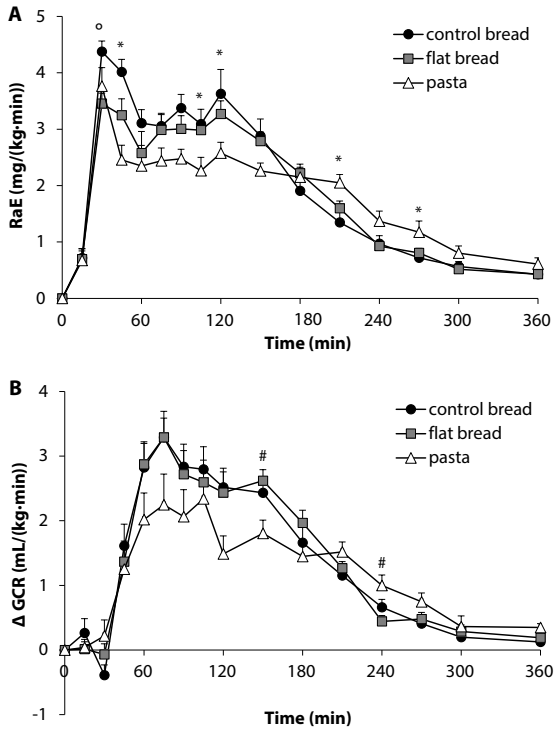

Figure 4. *Mean (± SEM) of (A) RaE and (B) changes from baseline in GCR, after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). There was a significant time × treatment interaction for RaE (P < 0.0001) and GCR (P = 0.0002). *Significantly different between CB and PA per time point (after Benjamini Hochberg correction). °Significantly different between CB and FB per time point (after Benjamini Hochberg correction). #Significantly different between PA and FB per time point (after Benjamini Hochberg correction). GCR, glucose clearance rate, RaE, rate of appearance of exogenous glucose.*

Glucose kinetics

FB intake resulted in a slower RaE compared to CB at t = 30 min ($P < 0.005$), but was similar to CB in the later postprandial phase. The RaE was slower after PA compared with CB intake at several time points (**Figure 4** A), resulting in a lower 0-2 h iAUC ($P < 0.005$) (Table 2). In the later phase, the RaE after PA was higher compared to CB (t = 210 and 270 min, $P < 0.005$). The average peak values of RaE from FB and PA were also lower compared to CB (Table 2). The EGP was not significantly different after consumption of the meals (**Supplemental Figure 1**). The GCR after FB and CB showed a remarkable similarity, whereas after PA consumption, glucose was cleared from the circulation at a slower rate (Figure 4 B). The GCR after PA and FB consumption was different at t = 150 min (FB higher) and at t = 240 min (PA higher) ($P < 0.005$). No partial tests for iAUC were performed because of a lack of difference based on the F test.

Postprandial glucagon, incretin and CCK response

The postprandial glucagon responses were not significantly different between the test meals (**Supplemental Figure 2**). The average postprandial GIP response after FB consumption tended to be lower compared to CB and was similar compared to the response after PA intake (**Figure 5** A). However, no partial tests could be performed on iAUC data (Table 2; F test, $P = 0.16$). Looking at time points, a significant difference in GIP response after CB and PA consumption was found at t = 120

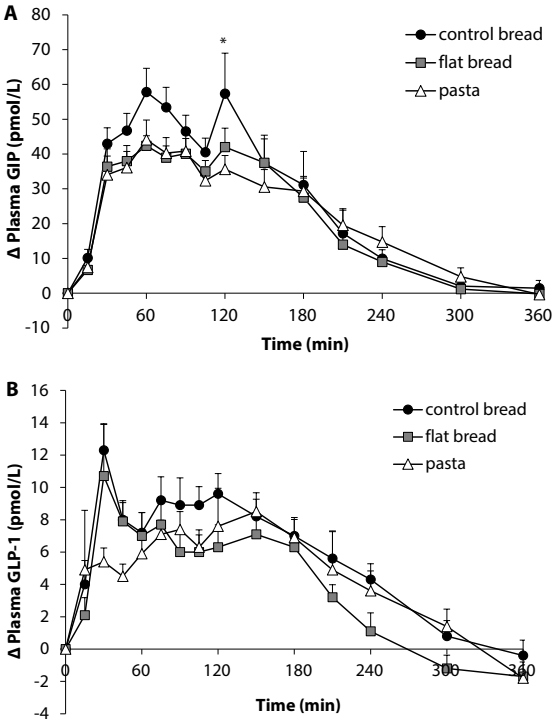

Figure 5. *Mean (± SEM) changes from baseline in (A) plasma GIP concentrations and (B) plasma GLP-1 concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). There was a significant time × treatment interaction for GIP (P = 0.0355), but not for GLP-1 (P = 0.0733). *Significantly different between CB and PA per time point (after Benjamini Hochberg correction). GIP, glucose-dependent insulinotropic polypeptide, GLP-1, glucagon-like peptide-1.*

min ($P < 0.005$).

The GLP-1 response showed a different pattern after PA compared to CB and FB consumption (Figure 5 B), especially around t = 30 min, where PA consumption did not evoke the sharp peak in GLP-1 concentrations observed after FB and CB consumption. However, time x treatment interaction failed to reach significance ($P = 0.0733$). The GLP-1 response to FB was similar compared to CB in the first hour, but tended to drop thereafter. The GLP-1 response after PA increased gradually, and reached a similar average increase from baseline compared to CB (t = 150 min). The iAUCs of GLP-1 responses were similar between meals (Table 2). The average postprandial CCK response was lower after PA consumption compared to CB between t = 0 and 180 min (0-2 and 0-6 h iAUC, $P < 0.005$), whereas the response to FB was intermediate (**Figure 6**).

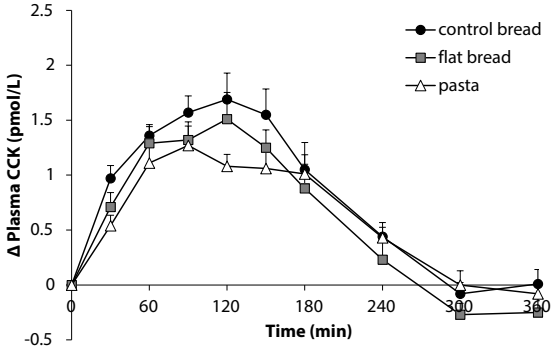

Figure 6. *Mean (± SEM) changes from baseline in plasma CCK concentrations after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). There was no significant time × treatment interaction (P = 0.4559). CCK, cholecystokinin.*

Postprandial bile acid response

The postprandial pattern of each individual (glycine- and taurine-) conjugated BA was identical, with the highest concentrations for GCDCA, followed by either GDCA or GCA. The unconjugated BAs (both primary and secondary) also responded in the same way within each person per test period. Therefore, by summing up concentrations, individual BAs were grouped as conjugated and unconjugated BAs, and together they formed the total BA response (**Figure 7** A). Except for the high peak at t = 30 min after FB, conjugated BAs after FB and PA showed a similar pattern, whereas CB consumption showed a biphasic pattern with a second peak around t = 120 min (Figure 7 C). Due to the great inter-individual differences in postprandial responses, F tests did not result in significant *P*-values.

$^{13}CO_2$ excretion in breath

$^{13}CO_2$ excretion in breath, reflecting the rate of oxidation of the ^{13}C-labeled substrate, was higher after CB compared to PA (t = 90 – 210 min, and 0-2 h iAUC, $P < 0.005$) and to FB (t = 120 – 180 min, and 0-2 and 0-6 h iAUC, $P < 0.005$) (Table 2, **Figure 8**). The $^{13}CO_2$ responses were similar after PA and FB consumption.

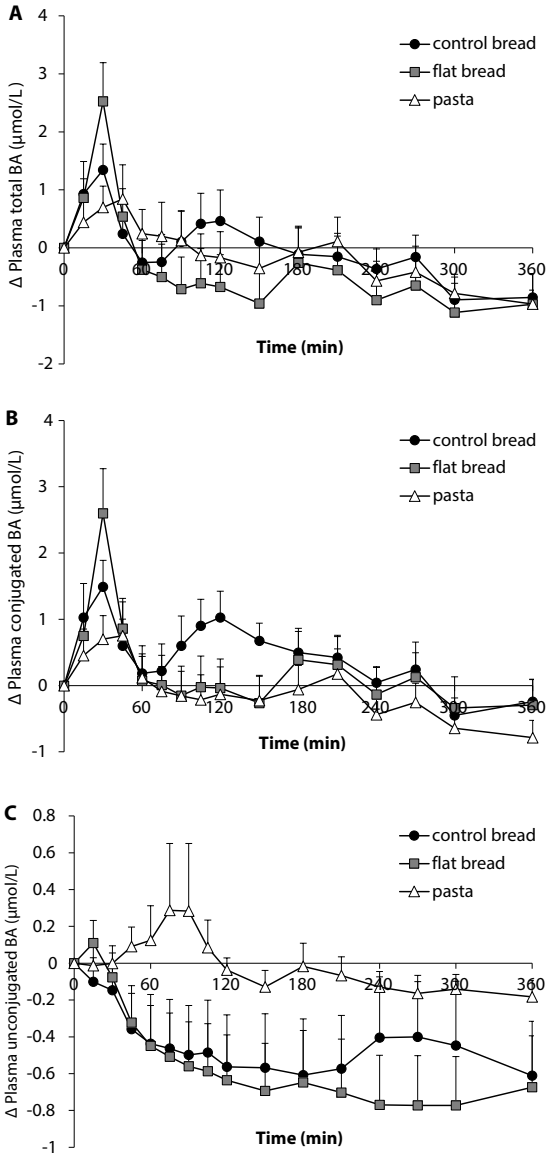

Figure 7. *Mean (± SEM) changes from baseline in (A) total plasma BA concentrations, (B) unconjugated BA concentrations and (C) conjugated BA concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). There were no significant time × treatment interactions. BA, Bile acids.*

Rated hunger, discomfort and liking of test meal

The subjective sensation of hunger, as determined hourly using a VAS, did not differ after consumption of CB, FB, and PA (**Supplemental Figure 3**), which might be due to a lack of power. Occasional mild complaints of flatulence were not meal type related. The VAS scores for liking (CB = 57, PA = 62, FB = 62) indicated that all meals were appreciated similarly by the subjects.

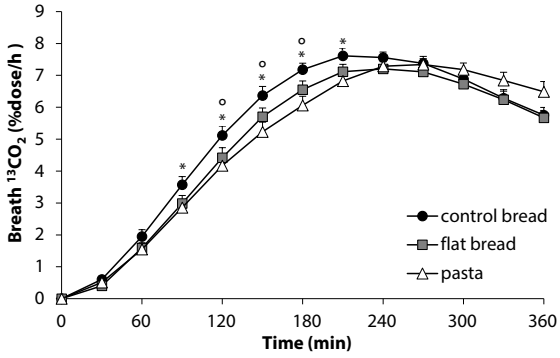

Figure 8. *Postprandial breath $^{13}CO_2$ response after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). There was a significant time × treatment interaction for breath $^{13}CO_2$ ($P < 0.0001$) *Significantly different between CB and PA per time point (after Benjamini Hochberg correction). °Significantly different between CB and FB per time point (after Benjamini Hochberg correction).*

Correlations

Correlations between several variables (all time points, 0-6 h) were determined. RaE and GIP correlated very well (CB r = 0.84; FB r = 0.81, PA r = 0.75), whereas RaE and GLP-1 correlated moderately for CB (r = 0.70) and FB (r = 0.69), but less for PA (r = 0.47). The correlation between GLP-1 and conjugated BA was moderate for CB (r = 0.60), but was lower for FB (r = 0.40), and PA (r = 0.28).

Insulin sensitivity

Post hoc analysis of possible difference in insulin sensitivity revealed that for the time period 0-3 h, there was a difference between PA and FB (t test, $P < 0.05$) **(Supplemental Table 2)**. A higher relative value indicates increased insulin sensitivity.

DISCUSSION

Because food structure may be important in determining starch digestibility and the metabolic response to a food product, we hypothesized that fiber-rich wheat bread with a compact structure would result in a more beneficial response compared to normal fiber-rich bread, and show similarities to fiber-rich wheat pasta with respect to postprandial glucose kinetics and associated processes. Therefore, we made compact flat breads (FB) without the use of a leavening agent and compared this to a yeast-leavened porous wheat bread (CB, control bread with open structure) and to pasta (PA, control wheat product with compact structure). All products had the same composition, but different food structures due to the processing conditions.

As confirmed by microscopy, XRT analysis (porosity) and density measurements, the structure of FB was indeed considerably more compact compared to CB (porosity CB 83% vs FB 47%, density CB 0.29 g/mL vs FB 0.47 g/mL). However, although no leavening agent was used in these breads, some air bubbles were present because of the mixing and baking procedure, resulting in a porosity of 47%. FB was, therefore, not as compact as pasta (density cooked PA 1.15 g/mL), which did not contain any air cells. Our pasta did show some porosity (3%), which was due to the addition of fiber resulting in small cracks in the pasta. These cracks, making the pasta structure less dense, might also

(partly) explain why this pasta did not result in a low glucose response (neither in the present nor the previous study (11)) as most often is the case for pasta as described in previous literature (36). From microscopy data FB appeared to contain less swollen starch granules. This can be explained by the very short baking process of FB, which leaves little time for complete swelling and gelatinization of the starch and is likely to decrease starch digestibility as well.

The *in vivo* digestibility of the products is reflected by the RaE. After FB consumption the RaE was lower compared to CB in the first hour postprandial (t = 30 and peak value), and comparable to the low PA response. However, although not significantly different, the RaE between 45 and 150 min postprandial was intermediate for FB compared to PA and CB, indicating that starch digestibility and/or oral processing, gastric emptying, etc was different between the meals. The *in vitro* digestibility data (Table 1) suggested that FB was most similar to CB, but contained some more resistant starch.

Despite the differences in RaE, the resulting total glucose response after consumption of the test meals was similar based on the iAUC. This corresponds to the findings in our previous study comparing CB and PA (11), although this time the peak value of glucose was somewhat lower for PA and also for FB compared to CB. The insulin response was, however, clearly lower after FB compared to CB, and was similar to the low insulin response after PA consumption, as observed previously (11).

GIP, one of the incretin hormones involved in insulin potentiation, responded very similarly after FB and PA consumption, whereas the increase was less pronounced than after CB. In previous studies, we showed that the RaE (influx rate of glucose from the meal) and the GIP response are in good agreement (10,11), which can be explained by the importance of the intestinal glucose transporter SGLT-1 in GIP secretion (37). The relation between glucose absorption and GIP release (dose-response) was also shown in several duodenal perfusion studies (38, 39). In the present study, this relationship between RaE and GIP was again demonstrated by the good correlation coefficients (CB r = 0.84, FB r = 0.81, PA r = 0.75). However, the obviously faster RaE from t = 45 min after FB as compared to PA (ns) was not reflected in the GIP response. It might be that the initial glucose influx rate is the most important factor in determining the extent of the GIP response, as the RaE at 30 min was the same after FB and PA and significantly lower than after CB in this early postprandial phase (t = 30 min FB, t = 45 min PA). In support of this, the initial rate of delivery of glucose to the duodenum was found to be important in determining the pattern of the incretin response (40). An initial rapid infusion rate (3 kcal/min) for 15 min and subsequent slower infusion rate of 0.71 kcal/min until t = 120 resulted in a high peak in plasma GIP and GLP-1 responses around 30 min, whereas the same amount of calories infused at a constant rate of 1 kcal/min resulted in a low response (40).

The initial high influx rate of glucose in our study, however, cannot explain the difference in GLP-1 response. We observed a sharp GLP-1 peak at t = 30 min after FB, which was very similar to that after CB, but absent after PA consumption. As the RaE indicated that the rate of glucose absorption was similar between PA and FB at around 30 min postprandially, the 'difference' in the initial GLP-1 peak between FB and PA cannot be explained by this mechanism. Although SGLT-1 plays a role in stimulating GLP-1 release to some extent (37, 41), other factors are capable of

stimulating GLP-1 secretion from L-cells as well. For instance, GLP-1 release, but not GIP release, was found to be influenced also by BAs via the BA receptor TGR5 in the small intestine (42). Interestingly, we observed a similar pattern when comparing GLP-1 and plasma BA concentrations after FB and PA, mainly with respect to the first postprandial hour. Conjugated BA peaked after FB around 30 min postprandial, whereas PA consumption resulted only in a modest increase in BAs. It could thus be speculated that the high conjugated BA peak after FB had an additional effect on GLP-1 secretion, thereby explaining the initial high GLP-1 peak. The reason for this 'difference' in plasma BA peak is not evident. CCK is known to stimulate gall bladder contraction and thus BA release(43), but the CCK response in our study did not correspond well with the BA concentrations.

Insulin is an important factor in glucose uptake into insulin sensitive tissues (via GLUT4 translocation), and therefore a low insulin response would be expected to result in a slow GCR. This was indeed observed in previous studies (11, 31). Interestingly, despite the lower insulin response (peak value and 0-2 h iAUC) to FB as compared to CB, the GCR after CB and FB consumption was almost identical. In addition, although insulin was very similar after PA and FB, the GCR was higher after FB compared to PA intake. Taken together, data from this study indicate either increased peripheral insulin sensitivity or increased insulin-independent glucose absorption after FB leading to augmented glucose disposal. Only speculations can be offered about the underlying mechanism. One factor involved in increasing insulin sensitivity and glucose disposal could be BAs. After FB consumption, the conjugated BA concentrations rose steeply (peak at t = 30 min), whereas no pronounced peak was observed after PA consumption. There are indications from rodent studies that BAs, via activation of their receptor FXR, could be involved in improving insulin sensitivity and increased glycogen storage (44-46). In addition, treatment of obese and diabetic mice with TUDCA resulted in normalization of hyperglycemia (within 10 days), restoration of systemic insulin sensitivity, and enhancement of insulin action in liver, muscle, and adipose tissue (47). Treatment of obese human subjects with TUDCA for 4 weeks improved muscle and hepatic insulin sensitivity (48). Although these are mainly longer term effects, it indicates the potency of BAs in regulating insulin sensitivity.

Previously we observed that a slow GCR (together with a slow RaE) resulted in a relatively 'high' glucose response after pasta consumption (11). Therefore, with an increased GCR after FB compared to PA, total glucose concentrations after FB would be expected to be lower. However, the similar glucose responses after FB and PA may be explained by the somewhat higher RaE, and a slightly less suppressed EGP after FB consumption.

To summarize, consumption of a compact flat bread resulted in a more moderate postprandial response compared to CB intake based on glucose, insulin and GIP responses, resembling that of pasta, although RaE was only lower in the first hour. Interestingly, the GCR after FB was higher than expected based on the insulin response and seems to be due to insulin-independent glucose disposal or increased insulin sensitivity. The role (and regulation) of BA and possibly other factors stimulating GLP-1 release and affecting insulin sensitivity after ingestion of starchy food products needs further study.

CONCLUSION

The results of this study clearly show that the structure of bread can influence the postprandial metabolic response. A more compact bread structure, due to reduced porosity and/or air cell diameter, may be advantageous because of the lower peak glucose, insulin and GIP (ns) response, via a slower initial RaE after FB consumption. Therefore, the effect of bread processing on postprandial metabolism and related health aspects deserves further study.

ACKNOWLEDGEMENTS

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REFERENCES

1. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am. J. Clin. Nutr. 2002;76:274S-280S.
2. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA. 2002;287:2414-2423.
3. Zammit VA, Waterman IJ, Topping D, McKay G. Insulin stimulation of hepatic triacylglycerol secretion and the etiology of insulin resistance. J. Nutr. 2001;131:2074-2077.
4. Deacon CF, Ahren B. Physiology of incretins in health and disease. Rev. Diabet. Stud. 2011;8:293-306.
5. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Ørskov C, Ritzel R, Schmiegel WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am. J. Physiol. 1997;273:E981-988.
6. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J. Clin. Invest. 1998;101:515-520.
7. Drucker DJ. Glucagon-Like Peptides: Regulators of Cell Proliferation, Differentiation, and Apoptosis. Mol. Endocrinol. 2003;17:161-171.
8. Yip RGC, Wolfe MM. GIP biology and fat metabolism. Life Sci. 2000;66:91-103.
9. Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, et al. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. Nat. Med. 2002;8:738-742.
10. Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Englyst KN, Holst JJ, Stellaard F, Vonk RJ. The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic polypeptide concentrations in healthy men. J. Nutr. 2006;136:1511-1516.
11. Eelderink C, Schepers M, Preston T, Vonk RJ, Oudhuis L, Priebe MG. Slowly and rapidly digestible starchy foods can elicit a similar glycemic response because of differential tissue glucose uptake in healthy men. Am. J. Clin. Nutr. 2012;96:1017-1024.
12. Vinoy S, Normand S, Meynier A, Sothier M, Louche-Pelissier C, Peyrat J, Maitrepierre C, Nazare JA, Brand-Miller J, Laville M. Cereal processing influences postprandial glucose metabolism as well as the GI effect. J. Am. Coll. Nutr. 2013;32:79-91.
13. Rizkalla SW, Laromiguiere M, Champ M, Bruzzo F, Boillot J, Slama G. Effect of baking process on postprandial metabolic consequences: Randomized trials in normal and type 2 diabetic subjects. Eur. J. Clin. Nutr. 2007;61:175-183.
14. Juntunen KS, Niskanen LK, Liukkonen KH, Poutanen KS, Holst JJ, Mykkanen HM. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. Am. J. Clin. Nutr. 2002;75:254-262.
15. Juntunen KS, Laaksonen DE, Autio K, Niskanen LK, Holst JJ, Savolainen KE, Liukkonen KH, Poutanen KS, Mykkänen HM. Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread. Am. J. Clin. Nutr. 2003;78:957-964.
16. Fardet A, Leenhardt F, Lioger D, Scalbert A, Remesy C. Parameters controlling the glycaemic response to breads. Nutr. Res. Rev. 2006;19:18-25.
17. Björck I, Liljeberg H, Ostman E. Low glycaemic-index foods. Br. J. Nutr. 2000;83 Suppl 1:S149-155.
18. Eelderink C, Noort MWJ, Sozer N, Koehorst M, Holst JJ, Deacon CF, Rehfeld JF, Poutanen K, Vonk RJ, Oudhuis L, et al. Difference in postprandial GLP-1 response despite similar glucose kinetics after consumption of wheat breads with different particle size in healthy men. Submitted.
19. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH. Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. Am. J. Clin. Nutr. 1999;69:448-454.
20. Verbeke K, Ferchaud-Roucher V, Preston T, Small AC, Henckaerts L, Krempf M, Wang H, Vonk RJ, Priebe MG. Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers. Eur. J. Clin. Nutr. 2010;64:678-684.

21. Ørskov C, Jeppesen J, Madsbad S, Holst JJ. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. J. Clin. Invest. 1991;87:415-423.

22. Lindgren O, Carr RD, Deacon CF, Holst JJ, Pacini G, Mari A, Ahren B. Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. J. Clin. Endocrinol. Metab. 2011;96:2519-2524.

23. Ørskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes. 1994;43:535-539.

24. Rehfeld JF. Accurate measurement of cholecystokinin in plasma. Clin. Chem. 1998;44:991-1001.

25. Vonk RJ, Stellaard F, Priebe MG, Koetse HA, Hagedoorn RE, De BS, Elzinga H, Lenoir-Wijnkoop I, Antoine JM. The 13C/2H-glucose test for determination of small intestinal lactase activity. Eur. J. Clin. Invest. 2001;31:226-233.

26. Vonk RJ, Hagedoorn RE, De Graaff R, Elzinga H, Tabak S, Yang YX, Stellaard F. Digestion of so-called resistant starch sources in the human small intestine. Am. J. Clin. Nutr. 2000;72:432-438.

27. Eelderink C, Moerdijk-Poortvliet TC, Wang H, Schepers M, Preston T, Boer T, Vonk RJ, Schierbeek H, Priebe MG. The glycemic response does not reflect the *in vivo* starch digestibility of fiber-rich wheat products in healthy men. J. Nutr. 2012;142:258-263.

28. Steele R, Wall JS, De Bodo RC, Altszuler N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am. J. Physiol. 1956;187:15-24.

29. De Bodo RC, Steele R, Altszuler N, Dunn A, Bishop JS. On the hormonal regulation of carbohydrate metabolism; studies with C14 glucose. Recent Prog. Horm. Res. 1963;19:445-488.

30. Tissot S, Normand S, Guilluy R, Pachiaudi C, Beylot M, Laville M, Cohen R, Mornex R, Riou JP. Use of a new gas chromatograph isotope ratio mass spectrometer to trace exogenous 13C labelled glucose at a very low level of enrichment in man. Diabetologia. 1990;33:449-456.

31. Schenk S, Davidson CJ, Zderic TW, Byerley LO, Coyle EF. Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am. J. Clin. Nutr. 2003;78:742-748.

32. Food and Agriculture Organization of the United Nations & World Health Organization. Carbohydrates in human nutrition. Report of a joint FAO/WHO expert consultation, 1998.

33. Cederholm J, Wibell L. Insulin release and peripheral sensitivity at the oral glucose tolerance test. Diabetes Res. Clin. Pract. 1990;10:167-175.

34. Priebe MG, Wang H, Weening D, Schepers M, Preston T, Vonk RJ. Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation. Am. J. Clin. Nutr. 2010;91:90-97.

35. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1-Correlation within subjects. BMJ. 1995;310:446.

36. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am. J. Clin. Nutr. 2002;76:5-56.

37. Moriya R, Shirakura T, Ito J, Mashiko S, Seo T. Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. Am. J. Physiol. Endocrinol. Metab. 2009;297:E1358-1365.

38. Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Smout AJPM, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. Am. J. Physiol. Endocrinol. Metab. 2007;293:E743-753.

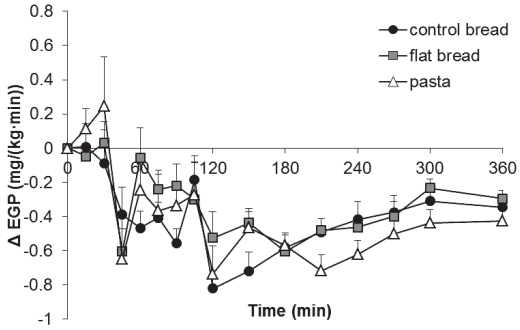
39. Trahair LG, Horowitz M, Rayner CK, Gentilcore D, Lange K, Wishart JM, Jones KL. Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. J. Clin. Endocrinol. Metab. 2012;97:844-851.

40. O'Donovan DG, Doran S, Feinle-Bisset C, Jones KL, Meyer JH, Wishart JM, Morris HA, Horowitz M. Effect of

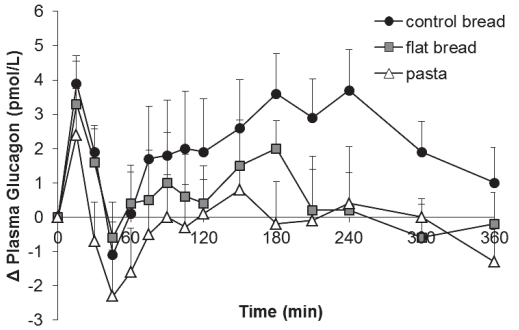
variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. J. Clin. Endocrinol. Metab. 2004;89:3431-3435.

41. Gorboulev V, Schurmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, et al. Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes. 2012;61:187-196.
42. Parker HE, Wallis K, le Roux CW, Wong KY, Reimann F, Gribble FM. Molecular mechanisms underlying bile acid-stimulated glucagon-like peptide-1 secretion. Br. J. Pharmacol. 2012;165:414-423.
43. Rehfeld JF. Clinical endocrinology and metabolism. Cholecystokinin. Best Pract. Res. Clin. Endocrinol. Metab. 2004;18:569-586.
44. Cariou B, van Harmelen K, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, Caron S, Torpier G, Fruchart JC, Gonzalez FJ, et al. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. J. Biol. Chem. 2006;281:11039-11049.
45. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM, Edwards PA. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc. Natl. Acad. Sci. U. S. A. 2006;103:1006-1011.
46. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. J. Clin. Invest. 2006;116:1102-1109.
47. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science. 2006;313:1137-1140.
48. Kars M, Yang L, Gregor MF, Mohammed BS, Pietka TA, Finck BN, Patterson BW, Horton JD, Mittendorfer B, Hotamisligil GS, et al. Tauroursodeoxycholic Acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. Diabetes. 2010;59:1899-1905.

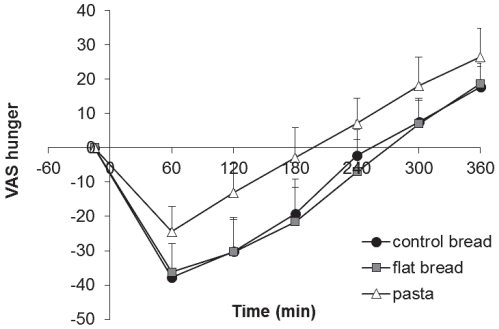
SUPPLEMENTARY FILES



Supplemental Figure 1. *Mean (± SEM) changes from baseline of EGP, after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). EGP, endogenous glucose production.*



Supplemental Figure 2. *Mean (± SEM) changes from baseline in plasma glucagon concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10).*



Supplemental Figure 3. *Mean (± SEM) changes from baseline in subjective rating of hunger using VAS after ingestion of 138 g ^{13}C-enriched control bread (●), 119 g ^{13}C-enriched flat bread (■), and 127 g ^{13}C-enriched pasta (Δ) in healthy men (n=10). VAS, visual analogue scale.*

Supplemental Table 1. *Image analysis parameters from 3D XRT data*[1]

	Average cell wall thickness (µm)	Average cell diameter (µm)	Porosity (%)
Control bread	143 ± 7[a]	900± 143[a]	83± 3[a]
Flat bread	278 ± 22[b]	476 ± 68[b]	47 ± 3[b]
Pasta	188 ± 36[c]	25 ± 1[c]	3 ± 1[c]

[1]Data are presented as means ± SD, n=3. Values followed by different letters in a column are significantly different (P < 0.05; LSD test). XRT, X-ray microtomography

Supplemental Table 2. *Post hoc analyses of insulin sensitivity after ingestion of 138 g ^{13}C-enriched control bread, 119 g ^{13}C-enriched flat bread and 127 g ^{13}C-enriched pasta (uncooked weight) using GCR and insulin data*[1]

	0-2h	0-3h	0-6h
CB	11.0[a]	18.4[a,b]	26.3[a]
FB	11.4[a]	**19.9[a]**	29.4[a]
PA	9.5[a]	**15.6[b]**	26.7[a]

[1]A higher relative value indicates increased insulin sensitivity. Values followed by different letters in a column are significantly different (P < 0.05; t-test). CB, control bread; FB, flat bread; PA, pasta.

Difference in postprandial GLP-1 response despite similar glucose kinetics after consumption of wheat breads with different particle size in healthy men

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ABSTRACT

PURPOSE: Underlying mechanisms of the beneficial health effects of low glycemic index (GI) starchy foods are not fully elucidated yet. We varied the wheat particle size to obtain fiber-rich breads with a high and low glycemic response and investigated the differences in postprandial glucose kinetics and metabolic response after their consumption.

METHODS: Ten healthy male volunteers participated in a randomized, crossover study, consuming ^{13}C-enriched breads with different structures; a control bread (CB) made from wheat flour combined with wheat bran, and a kernel bread (KB) where 85% of flour was substituted with broken wheat kernels. The structure of the breads was characterized extensively. The use of stable isotopes enabled calculation of glucose kinetics: rate of appearance of exogenous glucose, endogenous glucose production, and glucose clearance rate. Additionally, postprandial plasma concentrations of glucose, insulin, glucagon, incretins, cholecystokinin and bile acids were analyzed.

RESULTS: Despite the attempt to obtain a bread with a low glycemic response by replacing flour by broken kernels, the glycemic response and glucose kinetics were quite similar after consumption of CB and KB. Interestingly, the glucagon-like peptide-1 (GLP-1) response was much lower after KB compared to CB (iAUC, $P < 0.005$). A clear postprandial increase in plasma conjugated bile acids was observed after both meals.

CONCLUSIONS: Substitution of 85% wheat flour by broken kernels in bread did not result in a difference in glucose response and kinetics, but in a pronounced difference in GLP-1 response. Thus, changing the processing conditions of wheat for baking bread can influence the metabolic response beyond glycemia, and may thereby influence health.

INTRODUCTION

Consumption of foods with a low glycemic index (GI) instead of those with a high GI is considered beneficial for health, being associated with a decreased risk for the development of obesity, insulin resistance and type 2 diabetes (T2DM) (1-5), although this association is not always found (6). Based on results from a prospective cohort study, it was suggested that replacement of high GI bread by low GI bread in the diet may reduce the risk of developing T2DM (7). Possible explanations were, for instance, that consumption of a low GI diet might be associated with less weight gain (4) or decreased development of β-cell failure and insulin resistance (1, 3). However, the underlying mechanisms responsible for the beneficial effects need further study.

The postprandial glycemic response obviously depends on intestinal glucose influx, but is also influenced by suppression of endogenous glucose production (EGP) and increased glucose uptake in tissues, processes that are mainly regulated by the pancreatic hormones insulin and glucagon. The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), released postprandial from intestinal K- and L-cells, respectively, are known to potentiate the insulin response to a carbohydrate-rich meal, and are therefore important factors in glucose metabolism. Besides their role as incretin hormones, GIP is involved in fat metabolism (8), and GLP-1 is involved in decreasing gastric emptying rate (9), suppression of glucagon (10), as well as increasing satiety (11), and, in rodents, preserving β-cell function (12). Nowadays, bile acids (BAs) are also being recognized as signaling molecules in glucose metabolism. For instance, overexpression of the BA receptor TGR5 (13) and administration of taurocholic acid (TCA) (14) in mice was associated with elevated GLP-1 concentrations in response to an oral glucose tolerance test (OGTT). Although BAs are mainly released in response to fat ingestion, an increase in several plasma conjugated BAs was found after an OGTT (15, 16). Thus BAs, as well as (indirectly) the gastrointestinal hormone cholecystokinin (CCK) which stimulates gall bladder contraction, might also play a role in glucose metabolism after consumption of a carbohydrate-rich meal.

We were, therefore, interested in studying both the postprandial glucose kinetics as well as the metabolic responses to bread with a high and a low glycemic response, using wheat particle size as a variable factor. Postprandial glucose responses to wheat products prepared with different flour or grain particle size have been shown to decrease with increasing particle size (17), likely explained by decreasing the rate of amylolysis. Replacement of finely ground wheat flour by increasing the proportion of cracked wheat in bread (to 50 and 75%) also resulted in a reduction in GI (18).

In this crossover study, we investigated the metabolic effects of consumption of two ^{13}C-labeled wheat breads, with the same overall composition, only varying in structure; a bread prepared with flour and 85% broken wheat kernels and a control bread made from wheat flour combined with wheat bran, to obtain a similar dietary fiber content in both breads. We hypothesized that, due to the variation in particle size, the glycemic response, the underlying glucose kinetics, and possibly other factors involved in glucose metabolism would be different between both breads.

SUBJECTS AND METHODS

Subjects

Ten healthy men [age 24 ± 0.6 y, BMI 22 ± 0.2 kg/m^2 (mean ± SEM)] were recruited. The main criteria for exclusion were use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the 'Beoordeling Ethiek Biomedisch Onderzoek' foundation, Assen, The Netherlands. Each subject gave written informed consent for the study. This trial was registered at trialregister.nl as NTR3020.

Experimental design

In the overall study design, addressing two different research questions, 4 different wheat-based products were tested. To increase clarity and be able to focus on the results of one of these questions, only two meals are described in this paper. A description of the other products and the results can be found elsewhere (19). The study was performed in a randomized, crossover manner, with at least 1 wk between each study day. The subjects were asked to refrain from consuming foods naturally high in ^{13}C, like cane sugar, corn products and pineapple, for 3 d preceding the experiments and from alcohol consumption and strenuous exercise for 24 h before each study day. Food intake on the day before each study day was individually standardized. To minimize variation, a standard evening meal was provided at the commercial research facility (QPS Netherlands B.V.), where the participants stayed overnight. In the evening, a venous catheter was inserted in each forearm for blood collection and for infusion of the tracer D-[6,6-$^2H_2$]glucose (98% ^2H atom percent excess, Isotec) (20, 21). Subjects fasted overnight, but were allow ed to drink water. In the morning (t = -122 min), a bolus of 26.7 mL D-[6,6-$^2H_2$]glucose solution (80 × 0.07 mg/kg body weight) was injected within 2 min, and a continuous infusion of 0.07 mg/kg body weight D-[6,6-$^2H_2$]glucose per min was started (t = -120 min) and maintained for 8 h (until t = 360 min). The bolus amount was 80 times the infusion rate over 1 min, according to the paper of Tissot et al. (20). The infusion rate over 1 min was 0.07 mg/kg BW (21), to reach a good steady state. So the continuous infusion contained 4.2 mg/kg BW per 60 min, administered at a desired infusion rate of 20 mL/hour, resulting in the concentration of 0.21 mg/mL/kg BW. The bolus had the same concentration, and therefore, to administer 80 x 0.07 mg/kg BW an amount of 26.7 mL was administered.

Two hours after the start of the infusion the test meal was ingested (t = 0 min). **Figure 1** shows a simplified time line of each study day. Water (150 mL) was provided hourly, starting at t = 120 min. During the study period physical activity was limited.

Test meals

Two types of wheat bread were prepared (TNO, Zeist, The Netherlands), with the same composition, but a different structure, due to different milling- and bread making processes; a kernel bread (KB) prepared with flour and 85% broken wheat kernels, and a control bread (CB) made from wheat flour,

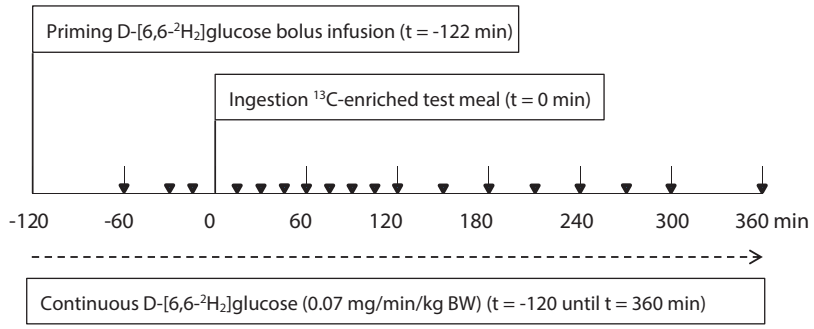

Figure 1. *Simplified schematic timeline of each study day. Blood was collected at 18 time points, indicated by ▼. Also, several breath and urine samples were collected during each study day.*

combined with wheat bran, to obtain the same dietary fiber content as in the KB.

To provide the necessary ingredients, unlabeled (1.085 Atom % ^{13}C) wheat grains [T. *aestivum* var *Capo*, grown in Austria] were milled in different ways; by conventional roller-milling the wheat kernels were milled to obtain refined white flour and wheat bran, and broken wheat kernels were obtained using a breaker mill. For ^{13}C-enrichment of the products, ^{13}C-labeled wheat [*T. aestivum* var *Paragon* (1.359 Atom % ^{13}C)], cultured in a $^{13}CO_2$ enriched atmosphere, was used. A small part of the ^{13}C-labeled wheat kernels was milled to obtain a fine wholemeal wheat flour, and the other part was broken to obtain broken kernels that were similar to the unlabeled broken kernels.

CB was prepared with 1446 g unlabeled white wheat flour, 240 g ^{13}C-labeled wholemeal wheat flour, 314 g wheat bran, 1300 g water, 33.4 g yeast, 36 g salt, 3 g malt and 70 ppm ascorbic acid. After kneading, the dough was left to rise for 30 min, moulded, and left to rise for 60 minutes. KB was prepared with 264 g unlabeled white wheat flour, 36 g ^{13}C-labeled wholemeal wheat flour, 1496 g unlabeled broken wheat kernels and 204 g ^{13}C-labeled broken wheat kernels. All broken kernels were soaked in 1000 g water overnight at 10°C. The following day, the soaked kernels were mixed with the flour, 230 g water, 33.4 g yeast, 36 g salt, 3 g malt and 70 ppm ascorbic acid. The resulting dough was left to rise for 30 min, moulded, and left to rise for 50 minutes. Subsequently, the breads were baked for 30 min at 240 °C. Bread slices were stored at -20°C until use.

All test meals provided 50 g available carbohydrates; for the CB the portion size was 138 g and for the KB 137 g. The breads were consumed together with 10 g light margarine (4 g fat), 2 slices lean ham (5 g fat, 6 g protein) and 250 mL tap water within 20 min. We assume that the digestibility of starch from both test products is similarly affected by this addition.

Bread characterization

Starch, dietary fiber and moisture content were determined at Eurofins Analytico Food, The Netherlands. To quantify starch fractions (G_T, G_{RA}, G_{SA}, G_{TA} and RS) of the test meals *in vitro*, an adapted version of the Englyst method (22) was used (23).

Particle size distribution of the broken kernels was determined by sieve analysis in duplicate. Bread products volume (mL) was determined in triplicate by rapeseed displacement (AACCI Method 10-05.01(24)) and, together with the bread weight (g), the product overall specific volume (mL/g) and density (g/mL) were obtained.

Breads were further characterized using microscopy and X-ray microtomography (XRT, porosity). A detailed description of both techniques can be found in **Online Resource 1**.

Sample collection

Blood was collected into several blood collection tubes (BD Diagnostics): 2 mL Fluoride tubes (NaF) for glucose and bile acid measurements, 3 mL EDTA tubes [+ 30 µL DPP-4 inhibitor (Millipore)] for GIP, GLP-1, glucagon and insulin determination, and 3 mL Lithium/Heparin tubes (CCK). Three basal blood samples were collected (t = -60, -30, -15 min) and postprandial samples were drawn every 15 min for 2 h, every 30 min for an additional 3 h, and once after 6h. To obtain heparin plasma, blood was collected at t = -60, t = -15 min and then every 30 min for the first 3 h, and hourly for the last 3 h. After centrifugation (1300 × *g* for 10 min at 4°C), plasma aliquots were stored at –20°C (NaF and EDTA plasma) or –80°C (heparin plasma) until analysis.

Breath samples were collected by breathing through a straw into 10 mL Exetainer vials (Labco Limited). Two basal breath samples were collected (t = -30, t = -5 min), and after the test meal, a sample was taken every 30 min until t = 360 min.

To get an impression about the impact of the breads on appetite, subjects were asked to rate their feeling of appetite (hunger) using a visual analogue scale (VAS) at 15 min before and hourly after the test meal, although the number of subjects (n=10) is insufficient to detect differences in these subjective measures (25), At the same time points, their feeling and extent of discomfort (abdominal pain, flatulence, other complaints) was recorded (0 = no complaints, 3 = severe complaints). Shortly after consumption of the meal, the subjects scored (VAS) how tasty the meal was.

Measurement of plasma concentrations

Plasma glucose concentrations were measured on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi) using a glucose hexokinase method. The within- and between-run CV were ≤ 2%. The ARCHITECT® insulin assay (Abbott Laboratories) was used to determine insulin concentrations in plasma. The total CV of this chemiluminescent microparticle immunoassay was ≤ 7%. The glucagon assay was directed against the C-terminal of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin (26).

Total GIP was measured using the C-terminally directed antiserum (no. 80867) (27), which reacts fully with intact GIP (1-42) and the N-terminally truncated metabolite GIP (3–42). Total GLP-1 concentrations were determined as previously described (28), using a radioimmunoassay (antiserum no. 89390) specific for the C-terminal of the GLP-1 molecule and reacting equally with

intact GLP-1 and the primary (N-terminally truncated) metabolite. The glucagon and incretin assays have detection limits of < 2 pmol/L, and an intra-assay coefficient of variation of approximately 6%. CCK was measured using antiserum no. 92128, which binds the bioactive forms of CCK with equal potency without cross-reactivity with any gastrin (29). The detection limit of the CCK assay is 0.1 pmol/L, and the intra-assay coefficient of variation approximately 5%.

Measurement of plasma bile acids

Fasting and postprandial concentrations of 15 individual BAs were determined using LC/MS; the primary BAs [cholic acid (CA) and chenodeoxycholic acid (CDCA)], the secondary BAs [deoxycholic acid (DCA), ursodeoxycholic acid (UDCA) and lithocholic acid (LCA)], as well as their glycine (G, glyco-) and taurine (T, tauro-) conjugates. The lower limit of quantitation (LOQ) was 0.05 µM. Concentrations of TCA, LCA, GLCA, TLCA, TDCA, UDCA and TUDCA were below the LOQ. The intra- and inter-assay CV ranged from 1.6% to 11.3% and 4.4% to 13.3%, respectively. For sample preparation, 250 µl of internal standard solution was mixed with 25 µl plasma, and centrifuged at 15.900 x g for 10 min. The supernatant was transferred into a new vial, evaporated under nitrogen at 40°C, and reconstituted in 100 µl of 50% methanol. The solution was filtered with a 0.2 µm centrifugal filter at 2000 x g for 10 min. After this step, the samples (injection volume 10 µl) were ready for analysis, using two different LC-MS systems. A detailed description of the systems and settings can be found in **Online Resource 2.**

Analysis of isotopic enrichment (breath and plasma) and calculations

Analysis of ^{13}C abundance in breath CO_2 was performed using GC/IRMS (Delta Plus XL; Thermo Fisher Scientific) measuring the $^{13}C/^{12}C$ ratio versus the international standard Pee Dee Belemnite ($\delta^{13}C_{PDB,}$ in ‰).

Plasma sample preparation required for analysis of isotopic enrichment by GC (derivatization) is described in detail elsewhere (30, 31). ^{2}H enrichment was measured by GC/MS as previously described (30) and $^{13}C/^{12}C$ isotope ratio was measured using GC/C/IRMS as previously described (32), both with some modifications (33). Calculation of glucose kinetics was performed as previously reported (34).

Incremental areas under the curve (iAUC)

To determine differences in glucose kinetics and plasma glucose, insulin, glucagon, incretin, CCK and bile acid concentrations, the 0-2 and 0-6 h iAUCs were calculated as previously described (34).

Statistics

Human data are presented as means ± SEM, n = 10. Baseline-subtracted values are shown in the figures and were used in the analyses as well, for clarity, consistency and comparison of variables. The overall study involved testing of 4 test meals. From the statistical analysis of the full experiment,

we highlight only the comparisons involving the products of current interest. For parameters that result in individual points of a time curve, we fitted a model that accounts for the differences between the 10 subjects and the 4 occasions, the main effects of treatment and time, respectively, and the time × treatment interaction. Residual plots based on such a model were used to assess approximate normality and homogeneity of variances. Parameters that were not compatible with these assumptions were transformed before definitive analysis. Most variables were log-transformed, except for glucose (reciprocal values), VAS scores (angular transformation), RaE and $^{13}CO_2$ values (Poisson regression model on log scale).

We performed F-tests on time × treatment interaction effects to assess whether the four test meals gave rise to curves of a different shape. If the test resulted in a P value <0.05, we tested differences between the meals for each time point. If there were no statistically significant differences in shapes, we tested overall differences between the meals based on the above model without the time × treatment interaction.

To assess summary measures such as iAUC, we fitted a model accounting for the differences in the means of the 10 subjects and the 4 occasions, and the main effects of treatment. Transformed data were used whenever residual plots showed incompatibility with normality and homogeneity assumptions. We performed an F test to see whether there were differences between the meals. If this test resulted in a P value <0.05, pairwise comparisons among the meals were conducted.

A Benjamini-Hochberg correction was applied on P-values from all partial tests to correct for multiple comparisons, resulting in a set of differences in which at most 5% were falsely selected ($P < 0.0052$ for partial tests was considered significant). The analyses were performed with the software package GenStat, release 13. The within-subject relationship (correlation) between variables was tested by regression analysis according to the method of Bland and Altman (35). Test meal differences (e.g. density, porosity) were assessed using a Student's *t*-test; a *P*-value < 0.05 was considered significant. These analyses were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago).

RESULTS

Characterization of test meals

The breads (CB and KB, respectively) were comparable in amount of starch (36.2 and 36.4%), dietary fiber (7.6 and 8.3%) and moisture content (38.6 and 37.8%). *In vitro* quantification of starch fractions showed similar digestive characteristics for CB and KB when a sample from the total bread was analysed (**Table 1**).

A main difference between the two breads (**Figure 2a**) was their density; CB had a density of 0.29 ± 0.007 g/mL compared to 0.41 ± 0.003 g/mL for KB ($P < 0.05$). The difference in density was clearly visible in the product structure (Figure 2b). In the case of KB, 85% of the wheat material consisted of broken kernels. The majority (72.6%) of the broken kernels had a particle size between 1680-2800 µm as measured by pan sieving. Both visual evaluation as well as microscopy confirmed that the KB

Table 1. *In vitro quantification of starch fractions in the test meals*[1]

Starch fraction	Test meal/portion			
	CB Total	KB Total	KB Kernel	KB Crumb
		%		
G_T	100	100	100	100
G_{RA}	78.8	76.4	65.5	67.8
G_{SA}	11.8	13.6	15.9	16.3
G_{TA}	90.6	90.0	81.4	84.1
RS	8.4	9.0	16.7	14.3

[1]*CB, control bread; G_{RA}, rapidly available glucose (20 min); G_{SA}, slowly available glucose (20-120 min); G_T, total glucose (indicated as 100%); G_{TA}, total available glucose (120 min); KB, kernel bread, RS, resistant starch.*

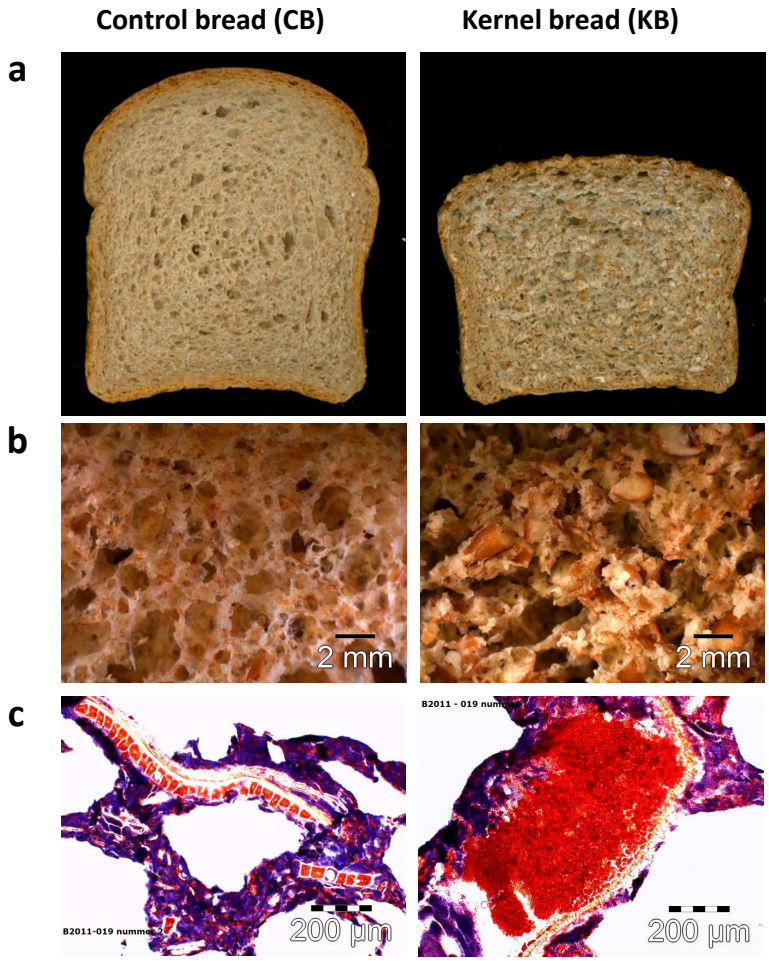

Figure 2. *Pictures of [13]C-enriched control and kernel bread: a, overview; b, stereo microscopy; c, light microscopy with starch (lugol; blue) and protein (Ponceau 2R; red) staining.*

structure consisted of broken wheat kernels with a particle size of 1 to several mm (Figure 2b). Light microscopy with starch (lugol; blue) and protein staining (Ponceau 2R; red) (Figure 2c) showed that CB had a porous structure and the thin cell walls were dominated by a continuous phase of starch granules in blue. In this matrix bran particles were clearly visible as clusters of red aleurone cells aligned on the outer side by pericarp layers. An apparent difference was the presence of broken wheat kernels in which no starch granules (blue) were observed (Figure 2c). Apparently, the matrix was governed by proteins (in red) embedding the starch granules.

A description of the porosity measurements of both breads using XRT can be found in Online Resource 1.

Postprandial glucose and insulin response

Postprandial glucose concentrations were similar after the consumption of CB and KB (**Figure 3a, Table 2**). The insulin response was lower after KB consumption compared to CB at t = 60 min (P = 0.002) (Figure 3b), which resulted in a 31% smaller iAUC (0-2 h) compared with CB intake (NS, P = 0.011).

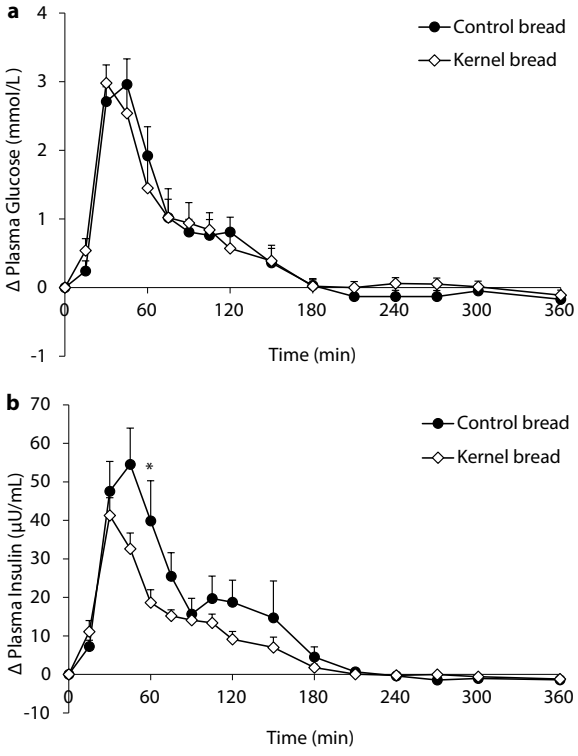

Figure 3. *Mean (± SEM) changes from baseline in (a) plasma glucose concentrations and (b) plasma insulin concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●) and 137 g ^{13}C-enriched kernel bread (◊) in healthy men (n = 10). There was no significant time × treatment interaction for glucose (P = 0.1773), but there was for insulin (P < 0.0001). *Significantly different between treatments per time point (after Benjamini-Hochberg correction).*

Table 2. *Indices reflecting the metabolic response after ingestion of 138 g ^{13}C-enriched control bread and 137 g ^{13}C-enriched kernel bread in healthy men[1]*

	Fasting values	Peak values	Time to peak (min)	iAUC (0-2 h)	iAUC (0-6 h)
Glucose (mmol/L)					
CB	5.1 ± 0.1	8.3 ± 0.3	40.5 ± 3.2	163.2 ± 20.5	197.0 ± 23.4
KB	5.0 ± 0.1	8.2 ± 0.3	34.5 ± 2.3	159.4 ± 30.1	195.4 ± 35.4
Insulin (µU/mL)					
CB	4.9 ± 0.5	69.9 ± 10.5	43.5 ± 3.5	3290.9 ± 528.8	4193.0 ± 936.6
KB	5.0 ± 0.5	48.7 ± 4.5	36.0 ± 2.4	2262.1 ± 205.4	2707.6 ± 292.9
Glucagon (pmol/L)					
CB	8.3 ± 1.0	15.8 ± 1.7	175.5 ± 33.4	272.9 ± 84.6	980.5 ± 163.4
KB	10.4 ± 1.2	16.8 ± 2.3	136.5 ± 38.2	207.1 ± 89.4	706.8 ± 199.9
GIP (pmol/L)					
CB	9.2 ± 1.8	84.9 ± 10.5	93.0 ± 13.6	4904.6 ± 426.7	9089.2 ± 1137.8
KB	12.4 ± 1.6	74.5 ± 11.0	84.0 ± 13.5	4494.8 ± 743.1	7169.4 ± 989.0
GLP-1 (pmol/L)					
CB	15.5 ± 1.4	30.9 ± 2.1	86.3 ± 15.7	949.9 ± 95.0	2019.9 ± 183.0
KB	18.3 ± 1.6	30.2 ± 1.8	76.5 ± 16.9	594.7 ± 102.4*	1167.0 ± 295.3*
RaT (mg/kg·min)					
CB	2.0 ± 0.1	6.5 ± 0.3	57.0 ± 14.5	39.1 ± 1.4	88.1 ± 1.6
Kb	2.0 ± 0.0	6.8 ± 0.3	36.0 ± 6.0	38.2 ± 1.1	85.1 ± 1.4
RaE (mg/kg·min)					
CB	0 ± 0	4.9 ± 0.2	66.0 ± 14.9	49.7 ± 1.6	95.9 ± 4.7
KB	0 ± 0	4.9 ± 0.2	42.0 ± 12.0	46.9 ± 1.7	89.1 ± 1.8
EGP (mg/kg·min)[2]					
CB	2.0 ± 0.1	0.9 ± 0.2	111.0 ± 12.1	43.4 ± 9.3	157.8 ± 26.7
KB	2.0 ± 0.0	0.9 ± 0.2	117.0 ± 23.7	40.8 ± 8.8	151.4 ± 24.3
GCR (mL/kg·min)					
CB	2.5 ± 0.1	6.4 ± 0.2	100.5 ± 12.5	15.6 ± 1.5	32.7 ± 1.7
KB	2.5 ± 0.1	6.1 ± 0.3	85.5 ± 12.3	15.5 ± 1.9	30.9 ± 2.9
$^{13}CO_2$ (%dose/h)					
CB	0 ± 0	7.7 ± 0.2	228.0 ± 9.2	4.3 ± 0.3	31.7 ± 0.7
KB	0 ± 0	7.2 ± 0.2*	237.0 ± 7.0	4.2 ± 0.2	30.0 ± 0.8
CCK (pmol/L)					
CB	0.5 ± 0.1	2.6 ± 0.3	115.5 ± 10.5	142.4 ± 5.7	295.0 ± 26.4
KB	0.6 ± 0.1	2.2 ± 0.1	126.0 ± 14.0	132.0 ± 13.6	243.0 ± 26.2
Total BA (µmol/L)					
CB	2.1 ± 0.4	4.3 ± 0.7	61.5 ± 19.7	93.0 ± 23.3	171.2 ± 40.9
KB	2.1 ± 0.6	4.2 ± 0.7	63.0 ± 21.1	80.3 ± 18.1	139.8 ± 42.8
Conjugated BA (µmol/L)					
CB	1.2 ± 0.3	3.6 ± 0.6	63.0 ± 19.3	110.8 ± 21.3	216.2 ± 43.4
KB	1.0 ± 0.3	3.3 ± 0.4	64.5 ± 20.7	108.7 ± 24.9	204.5 ± 43.8
Unconjugated BA (µmol/L)[2]					
CB	0.9 ± 0.3	0.2 ± 0.1	211.5 ± 38.0	47.7 ± 26.9	185.2 ± 96.9
KB	1.1 ± 0.5	0.2 ± 0.0	257.3 ± 41.3	60.2 ± 40.5	260.2 ± 160.9

*[1]Values are means ± SEM, n = 10. * Significantly different from control bread. BA, bile acid; CB, control bread; CCK, cholecystokinin; EGP, endogenous glucose production; GCR, glucose clearance rate; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; iAUC, incremental area under the curve; KB, kernel bread; RaE, rate of appearance of exogenous glucose; RaT, rate of appearance of total glucose.*

[2] Because EGP and unconjugated BAs were suppressed after the test meals, the nadir values and time to nadir are presented. Also, the area beneath baseline (dAUC) was calculated using mirrored data.

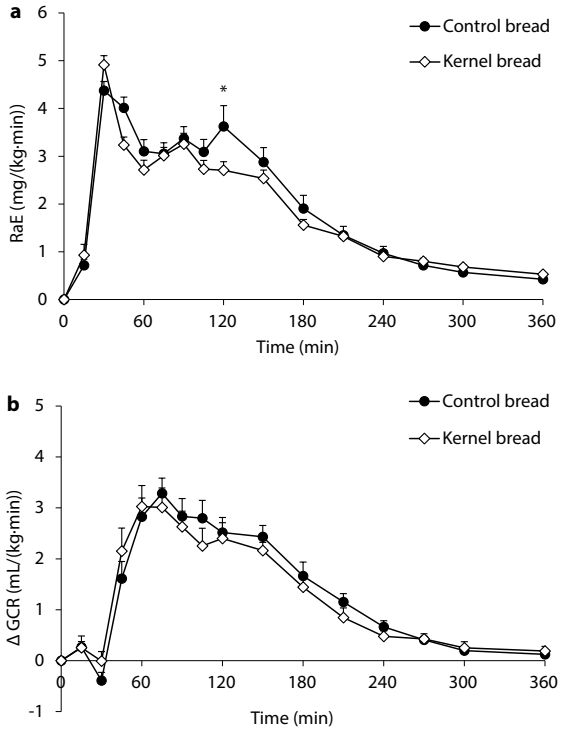

Figure 4. *Mean (± SEM) of (a) RaE and (b) changes from baseline in GCR, after ingestion of 138 g ^{13}C-enriched control bread (●) and 137 g ^{13}C-enriched kernel bread (◊) in healthy men (n = 10). There was a significant time × treatment interaction for RaE (P < 0.0001) and GCR (P = 0.0002). *Significantly different between treatments per time point (after Benjamini-Hochberg correction). RaE, rate of appearance of exogenous glucose, GCR, glucose clearance rate.*

Glucose kinetics

The RaE was similar after CB and KB consumption (**Figure 4a)**, except for t = 120 min, where RaE was higher after CB intake ($P = 0.005$). EGP was not significantly different after consumption of CB and KB. The rate at which glucose was cleared from the circulation (GCR) was also similar (Figure 4b, Table 2).

Postprandial incretin, glucagon, and CCK response

The average postprandial GIP response was somewhat higher after CB compared with KB intake (**Figure 5a**, Table 2), but a significant difference was only found at t = 120 min ($P < 0.005$). The average GLP-1 response was higher after CB compared to KB consumption at each time point (Figure 5b), resulting in a difference in the 0-2 and 0-6 h iAUC (Table 2, $P < 0.005$). Differences between time points could however not be determined, as time×treatment interaction failed to reach significance ($P = 0.073$).

The postprandial glucagon response appeared higher after CB consumption, but no partial test (CB-KB) could be performed for iAUC. Also, differences between meals at individual time points could not be tested, as there was no time×treatment interaction ($P = 0.9991$).

There was a slight increase in CCK concentrations in response to the test meals, which seemed somewhat lower after the KB from 90 min postprandial (**Figure 6a**, Table 2), although differences were not significant (iAUC 0-6 h, $P = 0.03$).

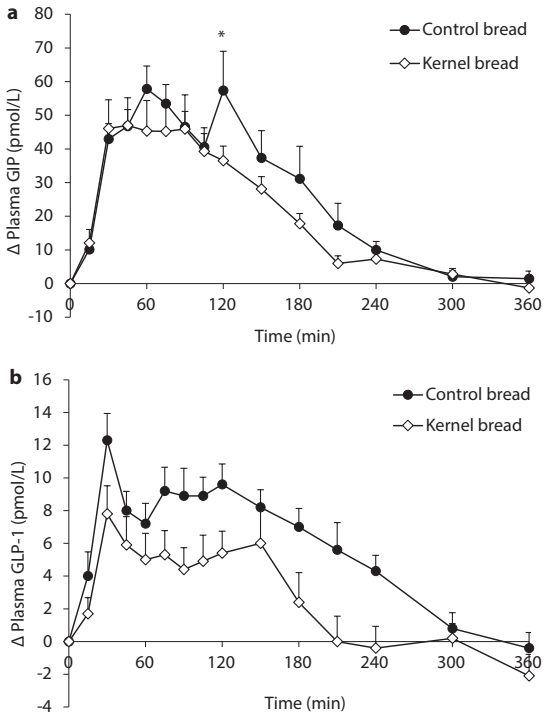

Figure 5. *Mean (± SEM) changes from baseline in (a) plasma GIP concentrations and (b) plasma GLP-1 concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●) and 137 g ^{13}C-enriched kernel bread (◊) in healthy men (n = 10). There was a significant time × treatment interaction for GIP (P = 0.036), but not for GLP-1 (P = 0.073). *Significantly different between treatments per time point (after Benjamini-Hochberg correction). GIP, glucose-dependent insulinotropic polypeptide, GLP-1, glucagon-like peptide-1.*

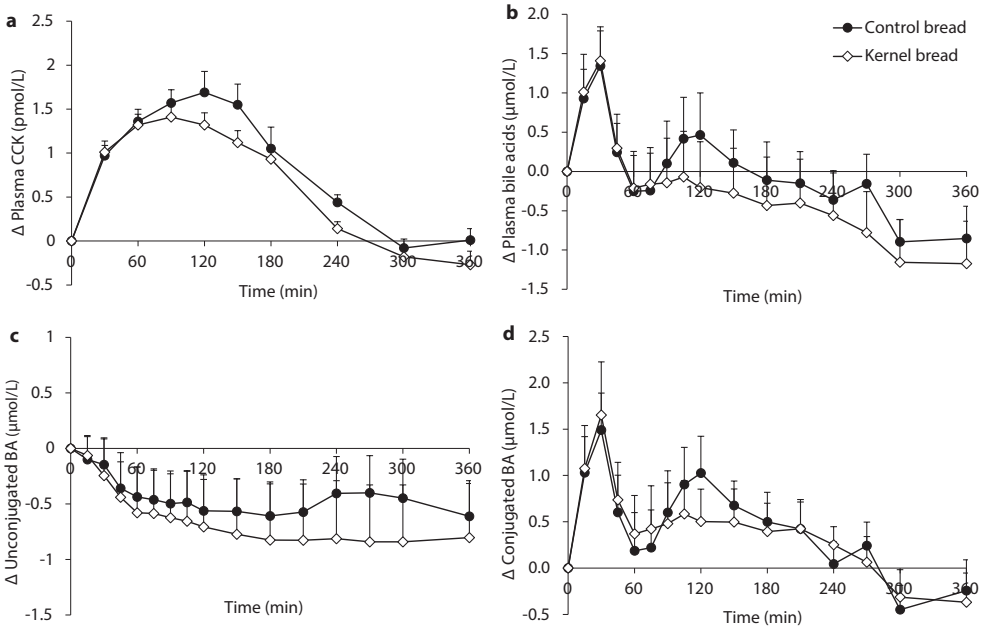

Figure 6. *Mean (± SEM) changes from baseline in (a) plasma CCK concentrations (b) total plasma BA concentrations, (c) unconjugated BA concentrations and (d) conjugated BA concentrations, after ingestion of 138 g ^{13}C-enriched control bread (●) and 137 g ^{13}C-enriched kernel bread (◊) in healthy men (n = 10). There were no significant time × treatment interactions. CCK, cholecystokinin, BA, Bile acid.*

Postprandial bile acid response

The postprandial pattern of glycine and taurine conjugated BAs in plasma was intra-individually identical, with the highest concentrations for GCDCA, followed by either GDCA or GCA. The unconjugated BAs (primary and secondary) also responded in the same way within each person per test period. Therefore, by summing up concentrations, individual BAs were grouped as conjugated and unconjugated BAs, and together they formed the total BA response (Figure 6b). Unconjugated BAs in plasma showed a decrease after test meal consumption (Figure 6c), whereas the conjugated BAs increased postprandial and peaked around t = 30 min (Figure 6d). After CB consumption, conjugated BAs showed a second peak around t = 120 min. Due to the great inter-individual variation in postprandial responses, F-tests did not result in significant *P*-values, so differences between CB and KB could not be tested.

$^{13}CO_2$ excretion in breath

$^{13}CO_2$ excretion in breath, reflecting the rate of oxidation of the ^{13}C-labeled substrate, was higher after CB consumption at t = 210 min ($P = 0.0014$). The time to peak was not different after the meals, but the peak value was significantly higher after CB consumption ($P = 0.005$) (Table 2).

Correlations

Correlations between several variables (all time points, 0-6 h; $P < 0.05$) were determined (**Table 3**). RaE and GIP were very well correlated, especially for CB (CB, r = 0.84; KB, r = 0.71). RaE and GLP-1 showed a moderate correlation (CB, r = 0.70; KB, r = 0.58). The correlation between GLP-1 and conjugated BAs was moderate for CB (r = 0.60), and lower for KB (r = 0.39). Total BAs with GLP-1 showed similar correlation coefficients (CB, r = 0.53; KB, r = 0.43).

Table 3. *Correlation coefficients relating the glucose, insulin, GIP, GLP-1, conjugated and total BA concentrations, and RaE after ingestion of 138g ^{13}C-enriched CB and 137g ^{13}C-enriched KB[1]*

	Glucose		Insulin		GIP		GLP-1		RaE		BA Con		BA Tot	
	CB	KB	CB	KB	CB	KB	CB	KB	CB	KB	CB	KB	CB	KB
Glucose														
Insulin	0.86	0.83												
GIP	0.57	0.56	0.55	0.63										
GLP-1	0.41	0.43	0.41	0.46	0.59	0.69								
RaE	0.73	0.71	0.66	0.76	0.84	0.71	0.70	0.58						
BA Con	0.21	0.24	0.18	0.41	0.34	0.27	0.60	0.39	0.41	0.38				
BA Tot	0.23	0.25	0.19	0.39	0.26	0.25	0.53	0.43	0.35	0.27	0.94	0.89		

[1]The within-subject relationship (correlation) between variables was tested by regression analysis according to the method of Bland and Altman[35], P < 0.05. BA Con, conjugated bile acids; BA Tot, total bile acids; CB, control bread; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; KB, kernel bread; RaE, rate of appearance of exogenous glucose.

Rated appetite, discomfort and liking of test meal

The subjective sensation of appetite (hunger), as determined hourly using a VAS, was similar after consumption of CB and KB (data not shown). Occasional mild complaints about flatulence were not meal type related. The liking of the test meal was rated by using a VAS (0 = not tasty, 100 = very tasty). The resulting scores (CB = 57, KB = 33) indicate that the KB was not well appreciated by the subjects.

DISCUSSION

This paper describes the glucose kinetics and metabolic effects in healthy men after consumption of control bread (CB) and 85% broken kernel bread (KB). We expected that consumption of KB would result in a low glycemic response due to slower digestion of starch in the larger wheat kernel particles (17, 18). However, the postprandial glycemic response did not differ after consumption of our breads. Although a similar glycemic response does not exclude a difference in *in vivo* starch digestibility due to possible differences in GCR (33, 34) or EGP, the present study found no pronounced differences in glucose kinetics. The RaE, reflecting intestinal glucose uptake, as well as the GCR and EGP, were similar after KB compared to CB. However, a clear difference in GLP-1 responses was observed after consumption of the test meals.

The use of intact grain kernels in bread formulations has been reported to reduce the glucose response by limiting starch gelatinization and forming a physical barrier for α-amylase, reducing amylolysis (36). For instance, the incorporation of 80% preboiled whole wheat kernels (37, 38) showed the expected lowering effect of incorporated kernels on the glycemic response. However, the use of intact kernels per se does not necessarily induce a low GI, as incorporation of oat kernels showed less effect on glycemia (38). Considering the palatability of the breads, we chose to incorporate broken wheat kernels and not intact kernels in KB, because the replacement of flour (50 and 75% (18)) with cracked wheat was also previously reported to result in a reduction in GI. The broken kernels were soaked overnight to prevent difficulties in chewing. Even though the broken kernels in KB were still clearly visible, during preparation of the dough the soaked kernels were thoroughly kneaded together with other ingredients, which might have further destructured the kernels, thus increasing starch accessibility to amylolysis. Breads were extensively characterized to document the impact of addition of broken kernels. The porosity, the average size of air cell diameter and air cell distribution was not significantly different between both breads. However, KB did have a higher density, which in itself is a characteristic that could give rise to a lower glycemic response (39). Moreover, the average air cell wall thickness of KB was significantly increased compared to CB, but these differences were apparently not large enough to evoke a difference in glucose response or kinetics.

Despite the similarities in the glycemic response, however, the GLP-1 response was much lower after KB consumption compared to CB (iAUC, $P<0.005$). Nutrient ingestion is the main stimulus to L-cells, which are described as an open type cell, enabling direct stimulation and release of GLP-1 by luminal contact (40). Although several mechanisms have been proposed with respect to glucose

sensing, in the early postprandial phase GLP-1 secretion seems to mainly involve the SGLT-1 glucose transporter in L-cells, similar to GIP secretion from K-cells (41, 42). In the present study, glucose transport across the intestinal membrane is reflected by the RaE, which correlated well with GLP-1 concentrations after the CB ($r = 0.70$, $P < 0.01$), but more moderately after the KB ($r = 0.58$, $P < 0.01$). However, the correlations of RaE with GIP (CB: $r = 0.84$ and KB: $r = 0.71$, $P < 0.01$), which are in accordance with our previous studies (31, 34), were stronger. The involvement of additional factors in stimulating GLP-1 release in the present study is thus likely.

We expected that due to the larger particle size in KB, more starch would reach the more abundant distal L-cells, resulting in an increased late-postprandial GLP-1 response. This was, for instance, seen in a study with slowly digestible starch, together with a prolonged influx of glucose (31). However, the GLP-1 response was prolonged after CB, but not after KB consumption, even though intestinal glucose uptake did not differ between our breads.

Moreover, with inhibition of SGLT-1 and in SGLT-1$^{-/-}$ mice, a late prolonged increase in GLP-1 after a glucose load was observed (43), besides the decreased GIP and GLP-1 responses in the early postprandial phase (41, 42). This indicates that the late postprandial GLP-1 response might not require glucose absorption via SGLT-1 (43). Because L-cells are densely distributed in the distal small intestine and colon, it might be that only the presence of still unabsorbed carbohydrates is important in stimulating GLP-1 in the late postprandial phase, for instance via other glucose-sensing mechanisms.

A prolonged postprandial GLP-1 response was also observed when sucrose digestion was delayed by acarbose (44, 45), which was linked to a simultaneous increase in breath hydrogen after 60-120 min, related to fermentation of sucrose. Another explanation for GLP-1 secretion in the late postprandial phase is therefore the involvement of short chain fatty acids (SCFAs), produced during fermentation of unabsorbed carbohydrates or fiber by the intestinal microbiota, and known to stimulate GLP-1 secretion as well (46). Although fermentation of solid food may be expected in a later phase, in a previous study using ^{13}C-labeled barley, ^{13}C-labeled SCFAs in plasma were detected within 3 h after ingestion (23). Increased SCFA formation after CB may be expected due to the presence of finer wheat bran (47).

In addition to nutrients and SCFAs, BAs are also able to potentiate GLP-1 release via activation of the BA receptor TGR5 (13,14, 48). Besides *in vitro* and animal data, in healthy humans, jejunal infusion with TCA and glucose increased GLP-1 concentrations (49). In our study, conjugated bile acids in plasma increased, whereas the unconjugated BAs decreased after bread consumption. These observations are in agreement with those of others made after an OGTT (50, 51). However, to the best of our knowledge, so far the differences in BA responses after various starchy food products have not yet been investigated. After KB consumption, the total BA response tended to be lower compared to CB (from t = 90 min), but differences were not significant and large inter-individual variations were observed. CCK, involved in regulating gall bladder contraction and thus BA release, showed only a slight increase in response to the test meals, which also tended to be somewhat

lower for KB between 90 and 180 min postprandial. However, an apparent difference between CCK and BA response patterns was the peak in BA concentration during the first 60 min, which was not detected in the CCK response in the present study.

We found a moderate correlation between GLP-1 and total BAs (CB r = 0.53, KB r = 0.43) and GLP-1 and conjugated BAs (CB r = 0.60, KB r = 0.39), indicating that there may be a relationship between the BA and GLP-1 response. In agreement, another study found a correlation of GLP-1 with mainly glycine-conjugated BAs and total BAs after ingestion of a mixed meal (52). The correlation coefficients for KB were clearly lower compared to CB, and as mentioned earlier, lower values for KB were also observed when correlating RaE with GIP and GLP-1. Because the interactions are likely to happen in the intestine, it might be that the more preserved kernel structure in KB resulted in less accurate glucose-sensing or interfered in BA-binding by the K- and/or L-cells.

It should also be kept in mind that the BAs measured in the systemic circulation do not necessarily directly reflect what happens in the intestine as, for instance, reabsorption of BAs might be influenced by food components. Fiber was found to bind BAs to a certain extent (53) and with respect to bran, especially more finely milled wheat bran was suggested to have higher BA binding capacity (54). Thus, the finer wheat bran fiber in CB could have bound more BAs, preventing reabsorption and resulting in higher exposure of the L-cells to BAs in the distal small intestine and colon, thereby contributing to the higher late GLP-1 response after CB. In support of this, it was shown in rodents that BA sequestrant administration could increase fecal BAs, stimulate TGR5 on L-cells located in the colon, and increase postprandial GLP-1 concentrations (55). Moreover, rectal infusion of TCA in obese T2DM patients resulted in a pronounced, dose-dependent GLP-1 response (56).

More research is necessary to understand the complex regulation of postprandial GLP-1 stimulation and the possible role of BAs. Therapies that increase BAs (or mimetics) in the distal bowel as a means of increasing endogenous GLP-1 concentrations have been suggested as novel treatments of T2DM and obesity (56). Several other approaches to increase GLP-1 action are being intensively studied for their potential therapeutic use. Furthermore, because of the beneficial effects in T2DM patients, treatment with GLP-1 analogs was also proposed for people with pre-diabetes to prevent progression to T2DM (57, 58). Pharmaceutical intervention might, however, have downsides, and increasing endogenous GLP-1 concentrations by nutritional means would therefore be interesting

Some potential limitations of this study should be considered. The breads were frozen after baking, to keep them fresh during the whole study period (4-week). Differential effects on digestibility of starch in the control or kernel bread cannot be excluded. Furthermore, we did not measure the protein content of the products. Protein can affect the blood glucose response, by influencing the insulin response independent of carbohydrates. However, we expected the protein content to be very similar, as both breads were made from the same wheat type and also had the same starch content.

To conclude, the substitution of 85% wheat flour by broken kernels in bread did not result in

any difference in glucose response and kinetics, but did elicit a pronounced difference in GLP-1 responses. Because of the complex regulation of GLP-1 release, several factors could simultaneously play a role after consumption of food products such as bread. Plasma bile acids showed a pronounced response after the breads, which might play a role in GLP-1 stimulation.

Our findings show that the glycemic response is not the only parameter which can determine health effects of starchy foods. The GLP-1 response is apparently largely independent from glucose kinetics and might be influenced by other characteristics of starchy food products. Therefore, our finding shows that bread processing technology can influence metabolic response beyond glycemia, which offers significant opportunities.

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REFERENCES

1. Ludwig DS (2002) The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 287:2414-2423
2. Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC (1997) Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care 20:545-550
3. Willett W, Manson J, Liu S (2002) Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 76:274S-280S
4. Thomas D, Elliott EJ, Baur L (2007) Low glycaemic index or low glycaemic load diets for overweight and obesity. Cochrane Database Syst Rev:Art. No.: CD005105
5. Thomas D, Elliott EJ (2009) Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. Cochrane Database Syst Rev:Art. No.: CD006296
6. Sluijs I, Beulens JW, van der Schouw YT, van der ADL, Buckland G, Kuijsten A, Schulze MB, Amiano P, Ardanaz E, Balkau B, Boeing H, Gavrila D, Grote VA, Key TJ, Li K, Nilsson P, Overvad K, Palli D, Panico S, Quiros JR, Rolandsson O, Roswall N, Sacerdote C, Sanchez MJ, Sieri S, Slimani N, Spijkerman AM, Tjonneland A, Tumino R, Sharp SJ, Langenberg C, Feskens EJ, Forouhi NG, Riboli E, Wareham NJ, InterAct consortium (2013) Dietary glycemic index, glycemic load, and digestible carbohydrate intake are not associated with risk of type 2 diabetes in eight European countries. J Nutr 143:93-99
7. Hodge AM, English DR, O'Dea K, Giles GG (2004) Glycemic index and dietary fiber and the risk of type 2 diabetes. Diabetes Care 27:2701-2706
8. Yip RGC, Wolfe MM (2000) GIP biology and fat metabolism. Life Sci 66:91-103
9. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Ørskov C, Ritzel R, Schmiegel WH (1997) Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 273:E981-8
10. Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W (1993) Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 36:741-744
11. Flint A, Raben A, Astrup A, Holst JJ (1998) Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 101:515-520
12. Drucker DJ (2003) Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. Mol Endocrinol 17:161-171
13. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K (2009) TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 10:167-177
14. Rafferty EP, Wylie AR, Hand KH, Elliott CE, Grieve DJ, Green BD (2011) Investigating the effects of physiological bile acids on GLP-1 secretion and glucose tolerance in normal and GLP-1R -/- mice. Biol Chem 392:539-546
15. Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, Carr SA, Thadhani R, Gerszten RE, Mootha VK (2008) Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol 4:214
16. Zhao X, Peter A, Fritsche J, Elcnerova M, Fritsche A, Haring HU, Schleicher ED, Xu G, Lehmann R (2009) Changes of the plasma metabolome during an oral glucose tolerance test: is there more than glucose to look at?. Am J Physiol Endocrinol Metab 296:E384-93
17. Holt SHA, Brand Miller JC (1994) Particle size, satiety and the glycaemic response. Eur J Clin Nutr 48:496-502
18. Jenkins DJA, Wesson V, Wolever TMS, Jenkins AL, Kalmusky J, Guidici S, Csima A, Josse RG, Wong GS (1988) Wholemeal versus wholegrain breads: Proportion of whole or cracked grain and the glycaemic response. Br Med J 297:958-960

19. Eelderink C, Noort MW, Sozer N, Koehorst M, Holst JJ, Deacon CF, Rehfeld JF, Poutanen K, Vonk RJ, Oudhuis L, Priebe MG (2015) The structure of wheat bread influences the postprandial metabolic response in healthy men. Food Funct 6:3236-3248

20. Tissot S, Normand S, Guilluy R, Pachiaudi C, Beylot M, Laville M, Cohen R, Mornex R, Riou JP (1990) Use of a new gas chromatograph isotope ratio mass spectrometer to trace exogenous ^{13}C labelled glucose at a very low level of enrichment in man. Diabetologia 33:449-456

21. Priebe MG, Wang H, Weening D, Schepers M, Preston T, Vonk RJ (2010) Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation. Am J Clin Nutr 91:90-97

22. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH (1999) Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. Am J Clin Nutr 69:448-454

23. Verbeke K, Ferchaud-Roucher V, Preston T, Small AC, Henckaerts L, Krempf M, Wang H, Vonk RJ, Priebe MG (2010) Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers. Eur J Clin Nutr 64:678-684

24. AACCI (2000) Method 10-05.01. Guidelines for measurement of volume by rapeseed displacement. In: Approved Methods of Analysis, 11th edition edn. AACC International, St. Paul, MN

25. Flint A, Raben A, Blundell JE, Astrup A (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. International journal of obesity 24:38-48

26. Ørskov C, Jeppesen J, Madsbad S, Holst JJ (1991) Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. J Clin Invest 87:415-423

27. Lindgren O, Carr RD, Deacon CF, Holst JJ, Pacini G, Mari A, Ahren B (2011) Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. J Clin Endocrinol Metab 96:2519-2524

28. Ørskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ (1994) Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 43:535-539

29. Rehfeld JF (1998) Accurate measurement of cholecystokinin in plasma. Clin Chem 44:991-1001

30. Vonk RJ, Stellaard F, Priebe MG, Koetse HA, Hagedoorn RE, De BS, Elzinga H, Lenoir-Wijnkoop I, Antoine JM (2001) The $^{13}C/^{2}H$-glucose test for determination of small intestinal lactase activity. Eur J Clin Invest 31:226-233

31. Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Englyst KN, Holst JJ, Stellaard F, Vonk RJ (2006) The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic polypeptide concentrations in healthy men. J Nutr 136:1511-1516

32. Vonk RJ, Hagedoorn RE, De Graaff R, Elzinga H, Tabak S, Yang YX, Stellaard F (2000) Digestion of so-called resistant starch sources in the human small intestine. Am J Clin Nutr 72:432-438

33. Eelderink C, Moerdijk-Poortvliet TC, Wang H, Schepers M, Preston T, Boer T, Vonk RJ, Schierbeek H, Priebe MG (2012) The glycemic response does not reflect the *in vivo* starch digestibility of fiber-rich wheat products in healthy men. J Nutr. doi: 10.3945/jn.111.147884

34. Eelderink C, Schepers M, Preston T, Vonk RJ, Oudhuis L, Priebe MG (2012) Slowly and rapidly digestible starchy foods can elicit a similar glycemic response because of differential tissue glucose uptake in healthy men. Am J Clin Nutr 96:1017-1024

35. Bland JM, Altman DG (1995) Calculating correlation coefficients with repeated observations: Part 1-Correlation within subjects. BMJ 310:446

36. Fardet A, Leenhardt F, Lioger D, Scalbert A, Remesy C (2006) Parameters controlling the glycaemic response to breads. Nutr Res Rev 19:18-25

37. Holm J, Bjorck I (1992) Bioavailability of starch in various wheat-based bread products: Evaluation of metabolic responses in healthy subjects and rate and extent of *in vitro* starch digestion. Am J Clin Nutr 55:420-429

38. Liljeberg H, Granfeldt Y, Bjorck I (1992) Metabolic responses to starch in bread containing intact kernels versus milled flour. Eur J Clin Nutr 46:561-575

39. Burton P, Lightowler HJ (2006) Influence of bread volume on glycaemic response and satiety. Br J Nutr 96:877-882

40. Eissele R, Goke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Goke B (1992) Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283-291

41. Moriya R, Shirakura T, Ito J, Mashiko S, Seo T (2009) Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. Am J Physiol Endocrinol Metab 297:E1358-65

42. Gorboulev V, Schurmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, Veyhl-Wichmann M, Srinivasan A, Balen D, Breljak D, Rexhepaj R, Parker HE, Gribble FM, Reimann F, Lang F, Wiese S, Sabolic I, Sendtner M, Koepsell H (2012) Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes 61:187-196

43. Powell DR, Smith M, Greer J, Harris A, Zhao S, DaCosta C, Mseeh F, Shadoan MK, Sands A, Zambrowicz B, Ding ZM (2013) LX4211 increases serum glucagon-like peptide 1 and peptide YY levels by reducing sodium/glucose cotransporter 1 (SGLT1)-mediated absorption of intestinal glucose. J Pharmacol Exp Ther 345:250-259

44. Qualmann C, Nauck MA, Holst JJ, Ørskov C, Creutzfeldt W (1995) Glucagon-like peptide 1 (7-36 amide) secretion in response to luminal sucrose from the upper and lower gut. A study using α-glucosidase inhibition (acarbose). Scand J Gastroenterol 30:892-896

45. Seifarth C, Bergmann J, Holst JJ, Ritzel R, Schmiegel W, Nauck MA (1998) Prolonged and enhanced secretion of glucagon-like peptide 1 (7-36 amide) after oral sucrose due to alpha-glucosidase inhibition (acarbose) in Type 2 diabetic patients. Diabet Med 15:485-491

46. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61:364-371

47. Jenkins DJA, Kendall CWC, Vuksan V, Augustin LSA, Li Y-, Lee B, Mehling CC, Parker T, Faulkner D, Seyler H, Vidgen E, Fulgoni III V (1999) The effect of wheat bran particle size on laxation and colonic fermentation. J Am Coll Nutr 18:339-345

48. Katsuma S, Hirasawa A, Tsujimoto G (2005) Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun 329:386-390

49. Wu T, Bound MJ, Standfield SD, Jones KL, Horowitz M, Rayner CK (2013) Effects of Taurocholic Acid on Glycemic, Glucagon-like Peptide-1, and Insulin Responses to Small Intestinal Glucose Infusion in Healthy Humans. J Clin Endocrinol Metab 98:E718-E722

50. Matysik S, Martin J, Bala M, Scherer M, Schaffler A, Schmitz G (2011) Bile acid signaling after an oral glucose tolerance test. Chem Phys Lipids 164:525-529

51. Ho JE, Larson MG, Vasan RS, Ghorbani A, Cheng S, Rhee EP, Florez JC, Clish CB, Gerszten RE, Wang TJ (2013) Metabolite profiles during oral glucose challenge. Diabetes 62:2689-2698

52. Roberts RE, Glicksman C, Alaghband-Zadeh J, Sherwood RA, Akuji N, le Roux CW (2011) The relationship between postprandial bile acid concentration, GLP-1, PYY and ghrelin. Clin Endocrinol (Oxf) 74:67-72

53. Story JA, Kritchevsky D (1976) Comparison of the binding of various bile acids and bile salts *in vitro* by several types of fiber. J Nutr 106:1292-1294

54. Kahlon T, de J. Berrios J, Smith G, Pan J (2006) *In vitro* bile acid binding capacity of milled wheat bran and milled extruded wheat bran at five specific mechanical energy levels. Cereal Chem 83:434-438

55. Harach T, Pols TW, Nomura M, Maida A, Watanabe M, Auwerx J, Schoonjans K (2012) TGR5 potentiates GLP-1 secretion in response to anionic exchange resins. Sci Rep 2:430

56. Adrian TE, Gariballa S, Parekh KA, Thomas SA, Saadi H, Al Kaabi J, Nagelkerke N, Gedulin B, Young AA (2012) Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in

obese type 2 diabetic volunteers. Diabetologia 55:2343-2347

57. DeFronzo RA, Abdul-Ghani M (2011) Type 2 diabetes can be prevented with early pharmacological intervention. Diabetes Care 34:S202-S209

58. Holst JJ, Deacon CF (2013) Is there a place for incretin therapies in obesity and prediabetes?. Trends Endocrinol Metab 24:145-152

ONLINE RESOURCE 1

Bread characterization – microscopy and X-ray microtomography

Methods - Microscopy

Stereomicroscopy was performed without sample preparation with an Olympus SZX-9 microscope connected to a DP-50 digital camera (settings Red 1.22; Green 1.22 and Blue 1.22). Light microscopy was performed with an Olympus BH-2 light microscope and the same camera type and settings. Sections of 10 µm thickness were cut in a cryostat-microtome at -26°C and applied to gelatin/glycerol coated slides. Sections were stained for 1 min with Lugol (KI-I2) to color the starch (blue) and for 10 minutes with 0.05% Ponceau 2R solution in 50% glycerol in water to color the protein (red). The excess of stain was rinsed with a minimum of water and sections were covered with glycerol.

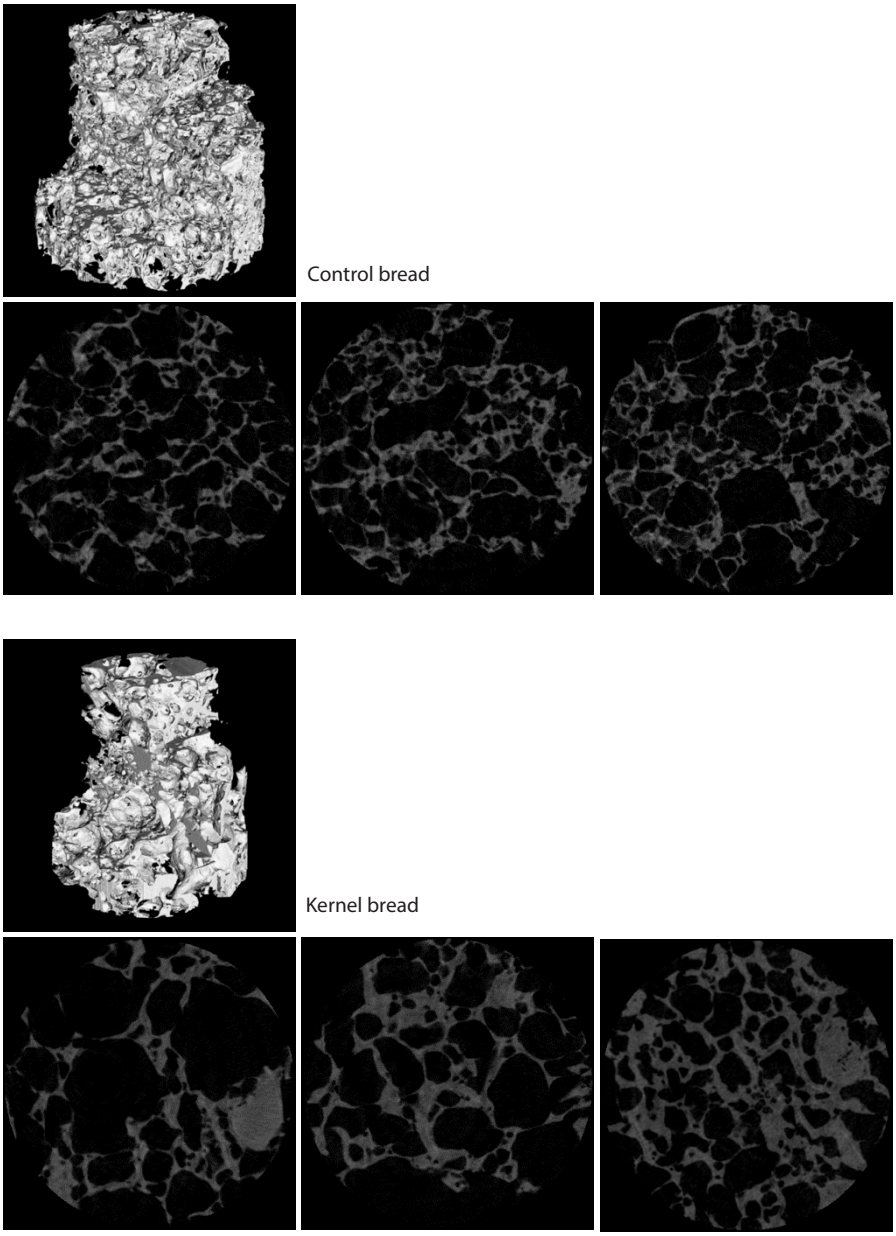
Methods - X-ray microtomography

To determine bread porosity, samples were scanned using a desktop X-ray microtomography (XRT) system (Model 1172, SkyScan, Aartselaar, Belgium) consisting of an X-ray tube, an X-ray detector and a CCD camera. The X-ray tube was operated at a voltage of 40 kV/250 µA to obtain optimum contrast between void (air cells) and matter (cell walls) according to a modified method [1]. A 12-bit cooled CCD camera (512 x 1024 pixels) was used to collect the X-ray data. Samples were rotated by a total of 180° during the scanning process with a pixel size of 24.31 µm to obtain optimum resolution, which gave a total scanning time of 18 min. The initial X-ray radiographs or raw images were obtained at every 0.7° of rotation. In order to avoid water migration during analysis, samples were sealed in stretch film. Samples were scanned in triplicate. After scanning, radiographs were loaded into NRecon reconstruction software (version 1.6.6). The software combined the images graphically into a 3-D object from which 2-D cross sectional images were taken. Before the reconstruction, the CS rotation feature was used to rotate the sample cross sections, making them parallel to the view window. Beam hardening correction was set to 40% in order to reduce the number of artefacts. Cell walls of the solid matrix appear grey, whereas air cells appear black. The reconstructed 2-D slices were then loaded into CTAn software (version 1.12, Skyscan, Belgium) to obtain the parameters of porosity, air cell wall thickness, and air cell diameter.

Results XRT

Although CB seemed more porous than KB, it was not significantly different (**Supplemental Figure 1, Supplemental Table 1**). Furthermore, the average air cell diameter was not statistically different between CB and KB (Supplemental Table 1). However, the predominant air cell diameter of CB ranged between 200-1000 µm, compared to 600-1200 µm for KB (**Supplemental Figure 2a**). Generally, KB had a more homogenous cell diameter distribution (Supplemental Figure 2a). Addition of broken wheat kernels resulted in thicker average cell walls in KB and had a major impact on the volumetric distribution of cell wall thickness. CB had a narrower range of cell wall thickness, mostly around 200

µm, whereas KB had a wider profile where the thickness went as high as 1500 µm (Supplemental Figure 2b) due to the presence of large kernel pieces. Average air cell wall thickness of KB (365 ± 33 µm) was more than double compared to CB (147 ± 11 µm) (Supplemental Table 1, $P < 0.05$).

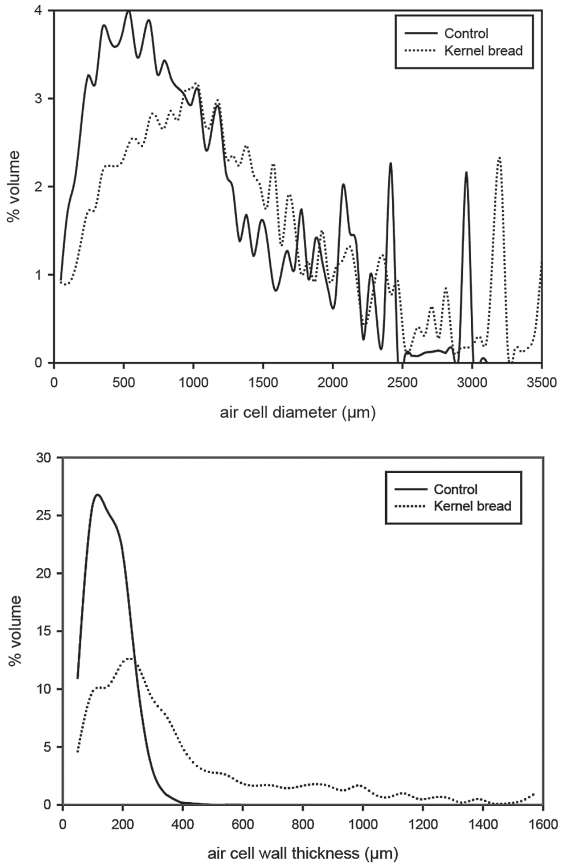


Supplemental Figure 1. *Reconstructed 3D- and selected representative 2D-images of control and kernel bread using X-ray microtomography.*

Supplemental Table 1. *Image analysis parameters from 3D XRT data*[1]

Sample	Average cell wall thickness (µm)	Average cell diameter (µm)	Porosity (%)
Control bread	143 ± 7[a]	900 ± 143[c]	83 ± 3[d]
Kernel bread	365 ± 33[b]	1065 ± 266[c]	73 ± 7[d]

[1]Values are means ± SD, n = 3. The values marked with different letter in a column are significantly different at 95% (P < 0.05) confidence level



Supplemental Figure 2. *Distribution profiles of (a) air cell diameter and (b) air cell wall thickness of 13C-enriched control bread (—) and kernel bread (·····).*

Reference

1. Sozer N, Dogan H, Kokini JL (2011) Textural properties and their correlation to cell structure in porous food materials. J Agric Food Chem 59:1498-1507

ONLINE RESOURCE 2

Supplemental Methods - Analysis of bile acids

Chemicals and reagents

Cholic acid (CA), tauro-cholic acid (TCA), glyco-chenodeoxycholic acid (GCDCA), ursodeoxycholic acid (UDCA), tauro-chenodeoxycholic acid (TCDCA), D4-chenodeoxychol acid (D4-CDCA) and D4-cholic acid (D4-CA) were purchased from Sigma-Aldrich (St. Lous, MO).

Lithocholic acid (LCA), deoxycholic acid (DCA), tauro-deoxycholic acid (TDCA), glyco-deoxycholic acid (GDCA), glyco-lithocholic acid (GLCA), chenodeoxycholic acid (CDCA), glyco-ursodeoxycholic acid (GUDCA), tauro-lithocholic acid (TLCA), glyco-cholic acid (GCA), and tauro-ursodeoxycholic acid (TUDCA) were purchased from Calbiochem.

D4-glyco-chenodeoxycholic acid (D4-GCDCA) and D4-glyco-cholic acid (D4-GCA) were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada).

D4-tauro-chenodeoxycholic acid (D4-TCDCA) and D4-tauro-cholic acid (D4-TCA) were purchased from Medical Isotopes (Pelham, NH).

HPLC-grade Methanol was purchased from Rathburn Chemicals (Walkerburn, Scotland).

Ammonium acetate and 25% ammonia were purchased from Merck (Darmstadt, Germany).

Instrumentation

Two different LC-MS systems were used for the analysis of the plasma samples.

System 1 consists of a SHIMADZU liquid chromatography (LC) system (SHIMADZU, Kyoto, Japan) coupled to an AB SCIEX API-3000 triple quadrupole mass spectrometer with electrospray ionization (ESI) source (AB SCIEX, Framingham, MA).

System 2 consists of an Agilent 1100 liquid chromatography (LC) system (Agilent, Santa Clara, CA) coupled to an AB SCIEX API-3200 triple quadrupole mass spectrometer with electrospray ionization (ESI) source (AB SCIEX, Framingham, MA).

The LC-MS systems are controlled by Analyst 1.6 software.

All chromatographic separations were performed with a XBridge ™ Shield RP18 column (100 mm x 2.1 mm, 3.5 µm) equipped with a XBridge ™ Shield RP18 guard column (10 mm x 2.1 mm, 3.5 µm) (Waters, Milford, MA).

Liquid chromatographic and mass spectrometric conditions

The mobile phase consisted of 20 mM ammonium acetate, adjusted to pH 8.0 with 25% ammonia (mobile phase A) and methanol (mobile phase B), at a total flow rate of 0.2 ml/min. The gradient profile is shown in Supplemental table 1.

Supplemental table 1. *Gradient profile*

Total time (min)	Flow rate (ml/min)	%A	%B
0.0	0.200	60	40
22.0	0.200	20	80
22.5	0.200	5	95
25.5	0.200	5	95
26.0	0.200	60	40
33.5	0.200	60	40

The injection volume of all samples was 10 µl.

The mass spectrometer parameters, such as gas pressure, voltage, temperature, etc., were optimized by infusing each analyte and the internal standards (IS) separate in a 50% MeOH solution via a Harvard pump 11 standard infusion syringe pump (Harvard Apparatus, South Natick, MA). All bile acids were detected in negative mode with the mass spectrometer source settings shown in Supplemental table 2 and 3.

Supplemental table 2. *Mass spectrometer source settings (system 1-API-3000) period 1 and 2*

Detection mode	MRM, negative mode
Resolution Q1	Unit
Resolution Q3	Unit
Nebulizer gas	12
Curtain gas	10
CAD gas	5
Ion spray voltage	-3000V
Temperature	450 °C
EP	-10
MR pause	5 msec

Supplemental table 3. *Mass spectrometer source settings (system 2-API-3200) period 1 and 2*

Detection mode	MRM, negative mode
Resolution Q1	Unit
Resolution Q3	Unit
Collision gas	10
CAD gas	10
Ion source gas 1	25
Ion source gas 2	30
Temperature	600 °C
Ion spray voltage	-4500V
MR pause	5 msec

The multiple reaction monitoring (MRM) transitions for each bile acid and internal standard, as well as their optimum MS parameters such as collision energy (CE), declustering potential (DP), focusing potential (FP), cell exit potential (CXP) and cell entrance potential (CEP) are shown in Supplemental table 4 and 5.

Sample preparation

For plasma samples, 250 µl of internal standard solution was added to 25 µl plasma, vortexed, and centrifuged at 15.900 x g for 10 min. The supernatant was transferred into a new vial, evaporated under nitrogen at 40 °C, and reconstituted in 100 µl of 50% methanol. The solution is now filtered with a 0.2 µm centrifugal filter at 2000 x g. After this step the samples are ready for analysis.

Supplemental table 4. *MRM settings system 1 (API-3000)*

Component	Q1 mass	Q3 mass	Dwell time (msec)	Declus-tering potential (DP)	Focus-sing potential (FP)	Collision energy (CE)	Cell exit potential (CXP)
UDCA	391.3	391.3	90	-96	-240	-5	-17
CA	407.2	407.2	90	-96	-140	-5	-17
D4-CA	411.2	411.2	90	-96	-140	-5	-17
GUDCA	448.4	74.0	90	-90	-310	-70	-15
GCA	464.2	74.1	90	-100	-240	-76	-13
D4-GCA	468.2	74.1	90	-100	-240	-76	-13
TUDCA	498.4	79.9	90	-101	-300	-102	-10
TCA	514.2	79.9	90	-100	-230	-100	-5
D4-TCA	518.2	79.9	90	-100	-230	-100	5
LCA	375.1	375.1	90	-101	-280	-5	-17
CDCA, DCA	391.3	391.3	90	-96	-240	-5	-17
D4-CDCA	395.3	395.3	90	-96	-240	-5	-17
GLCA	432.1	74.0	90	-101	-280	-68	-5
GCDCA, GDCA	448.4	74.0	90	-90	-310	-70	-15
D4-GCDCA	452.4	74.0	90	-90	-310	-70	-15
TLCA	482.2	79.8	90	-90	-340	-98	-5
TCDCA, TDCA	498.4	79.9	90	-101	-300	-102	-10
D4-TDCA	502.4	79.9	90	-101	-300	-102	-10

Supplemental table 5. *MRM settings system 2 (API-3200)*

Compo nent	Q1 mass	Q3 mass	Declus-tering potential (DP)	Cell entrance potential (CEP)	Collision energy (CE)	Cell exit potential (CXP)	Entrance Potential (EP)	Dwell time (msec)
UDCA	391.3	391.3	-130	-26	-13	-4	-4	90
CA	407.2	407.2	-28	-21	-14	-4	-12	90
D4-CA	411.2	411.2	-28	-21	-14	-4	-12	90
GUDCA	448.4	74.0	-118	-20	-63	-1	-10	90
GCA	464.2	74.1	-106	-23	-65	-1	-7	90
D4-GCA	468.2	74.1	-106	-23	-65	-1	-7	90
TUDCA	498.4	79.9	-23	-21	-83	-1	-5	90
TCA	514.2	79.9	-39	-25	-121	-1	-4	90
D4-TCA	518.2	79.9	-39	-25	-121	-1	-4	90
LCA	375.1	375.1	-123	-18	-13	-6	-7	90
CDCA	391.3	391.3	-134	-19	-13	-5	-10	90
DCA	391.3	391.3	-115	-19	-15	-5	-6	90
D4-CDCA	395.3	395.3	-134	-19	-13	-5	-40	90
GLCA	432.1	74.0	-100	-22	-58	-2	-4	90
GCDCA	448.4	74.0	-102	-20	-62	-1	-7	90
GDCA	448.4	74.0	-103	-20	-62	-1	-7	90
D4-GCDCA	452.4	74.0	-102	-20	-62	-1	-7	90
TLCA	482.2	79.8	-60	-22	-109	-1	-7	90
TCDCA	498.4	79.9	-146	-23	-111	-1	-7	90
TDCA	498.4	79.9	-153	-22	-115	-2	-7	90
D4-TDCA	502.4	79.9	-153	-22	-115	-2	-7	90

Metabolic profiling reveals differences in plasma concentrations of arabinose and xylose after consumption of fiber-rich pasta and wheat bread with differential rates of systemic appearance of exogenous glucose in healthy men

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ABSTRACT

BACKGROUND: The consumption of products rich in cereal fiber and with a low glycemic index is implicated in a lower risk of metabolic diseases. Previously, we showed that consumption of fiber-rich pasta compared with bread resulted in a lower rate of appearance of exogenous glucose and a lower glucose clearance rate quantified with a dual isotope technique, which was in accordance with a lower insulin and glucose-dependent insulinotropic polypeptide response.

OBJECTIVE: To gain more insight into the acute metabolic consequences of the consumption of products resulting in differential glucose kinetics, postprandial metabolic profiles were determined.

METHODS: In a crossover study, 9 healthy men [age 21±0.5 y, BMI 22±0.5 kg/m^2 (mean±SEM)] consumed wheat bread (132 g) and fresh pasta (119 g uncooked) enriched with wheat bran (10 %). A total of 134 different metabolites in postprandial plasma samples (at -5, 30, 60, 90, 120, and 180 min) were quantified by using a gas chromatography-mass spectrometry -based metabolomics approach (secondary outcomes). Two-factor ANOVA and advanced multivariate statistical analysis (partial least squares) were applied to detect differences between both food products.

RESULTS: 42 different postprandial metabolite profiles were identified, primarily representing pathways related to protein and energy metabolism, which were on average 8% and 7% lower after the men consumed pasta rather than bread, whereas concentrations of arabinose and xylose were 58% and 53% higher, respectively. Arabinose and xylose are derived from arabinoxylans, which are important components of wheat bran. The higher bioavailability of arabinose and xylose after pasta intake coincided with a lower rate of appearance of glucose and amino acids. We speculate that this higher bioavailability is due to higher degradation of arabinoxylans by small intestinal microbiota, facilitated by the higher viscosity of arabinoxylans after pasta intake than after bread intake.

CONCLUSION: This study suggests that wheat bran, depending on the method of processing, can increase the viscosity of the meal bolus in the small intestine and interfere with macronutrient absorption in healthy men, thereby influencing postprandial glucose and insulin responses. This trial was registered at controlled-trials.com as ISRCTN42106325.

INTRODUCTION

Metabolomics is increasingly proving its potential to monitor changes in metabolic profiles after nutritional interventions (1-3) or after consumption of single nutrients and food products (4-7) which ultimately can be used to elucidate the relationship between diet and health or disease.

Diets rich in cereal fiber and/or with a low glycemic index or glycemic load have been linked to a decreased risk of development of metabolic diseases, such as type 2 diabetes, cardiovascular diseases, and obesity (8-11). The underlying mechanisms are not fully understood; the lower glycemic response itself, the associated lower insulin response, the gastro-intestinal hormone response or fiber-associated bioactive compounds have been hypothesized to be involved in the reduced risk for these chronic lifestyle-related diseases (12-15).

To gain more insight into these mechanisms, we previously investigated changes in postprandial glucose kinetics as well as the insulin and glucose-dependent insulinotropic polypeptide (GIP) response after the ingestion of fiber-rich products with 'slowly' (pasta) and 'rapidly' (bread) digestible starch. We observed that the consumption of pasta compared with bread resulted in a slower rate of appearance of exogenous glucose (RaE), and lower postprandial insulin and GIP concentrations, but a similar glycemic response due to a slower glucose clearance rate (GCR) (16). This prompted us to perform detailed metabolic profiling to further examine differences in postprandial metabolism after consumption of these starchy food products.

METHODS

Subjects

Ten healthy men [age 21 ± 0.5 y, BMI 23 ± 0.6 kg/m^2 (mean ± SEM)] were recruited. The criteria for exclusion were use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the BEBO foundation, Assen, The Netherlands. Each subject gave written informed consent for the study. This trial was registered at controlled-trials.com as ISRCTN42106325. A part of the data obtained in this trial was previously published (16, 17).

Experimental design

A detailed description of the experimental design is given elsewhere (16, 17). The study was performed in a crossover manner, with at least 1 wk between each study day. Food intake on the day before each experiment was individually standardized. Subjects recorded their food intake using a diary the day before experiment 1 and consumed the same food on the day before experiment 2. A standard evening meal was provided at the commercial research facility (QPS Netherlands B.V.), where the subjects stayed overnight. Subjects fasted overnight, but were allowed to drink water. During the study period physical activity was limited.

Test meals

The wheat-based test meals were 132 g bread and 119 g fresh pasta (uncooked weight), prepared at TNO, Zeist, The Netherlands. The white wheat flour used for bread was from *T. aestivum* Kolibri/Ibis (ratio 70/30 and for pasta from *T. durum* de Cecco). Wheat bran (≈ 10 %, English Biscuit Wheat, Meneba) was added, resulting in 7 % dietary fiber in the final product, as described before (16, 17). Bread was stored at -20 °C until use. Pasta was freshly prepared, stored at 5 °C until use and cooked for 6.5 min in 2 L water before consumption. Both test-meals consisted of 50 g available carbohydrates and were consumed together with 10 g light margarine (4 g fat), 2 slices lean ham (5 g fat, 6 g protein) and 250 mL tap water within 20 min.

Sample collection

Blood was collected into 2 mL BD Vacutainer Fluoride tubes (BD Diagnostics). Three basal blood samples were collected (t = -60, t = -30, t = -5) and after the test meal samples were drawn every 15 min for 2 h and every 30 min for an additional 4 h. After centrifugation (1300 × g for 10 min at 4°C), plasma aliquots were stored at –20°C until analysis.

Metabolomics analyses of plasma samples

The GC-MS method used for the measurement of a broad range of metabolites was identical to the GC-MS method reported before (18) and applied with success on plasma samples as described previously (3, 6). In this study, 100 µl of plasma was extracted and further derivatized. Six samples per subject per test meal were analyzed: one baseline sample (t = - 5) and the samples collected at 30, 60, 90, 120 and 180 minutes after either bread or pasta consumption. Samples of only 9 subjects were used as one subject was excluded because he did not consume the pasta meal completely. The total of 108 plasma samples was analyzed in 4 different batches. The samples of one particular subject were analyzed within the same batch.

The 6 different time-points per subject were analyzed in randomized order. All the study samples were prepared and injected once. The performance of the applied metabolic profiling platforms was controlled by the frequent analysis of a quality control sample (QC), and method performance was monitored by 10 internal standards (including analogs and ^{2}H– and ^{13}C-labeled metabolites), as described previously (18, 19). The QC sample, prepared by pooling study samples, represents the biochemical diversity of the study samples and allows calculation of the analytical precision for all metabolites measured. Every batch contained 6 replicates of the QC samples, with exception of the last batch that contained 4 QC replicates. The replicate analyses of the QC samples were used to select the most suitable internal standard for each detected metabolite as well as to correct for any systematic errors, as described previously in more detail (19). After the above mentioned data correction steps, a total of 134 metabolites with known identity were quantified in the different plasma samples, of which a total of 100 metabolites had a RSD < 10 % and a total of 18 metabolites had a RSD between 10-20%. The data quality was considered to be good (comparable or of higher quality as compared to other human studies that used the same methodology (3, 6)).

In vitro analysis of arabinoxylan (AX), arabinose (A) and xylose (X) in flour, bran and starchy products

Because A and X were identified in the plasma samples with a distinct postprandial response profile, we analyzed the AX, A and X content of the wheat bran (English Biscuit Wheat) as well as of the hard (*T. durum* de Cecco) and the soft wheat flour (*T. aestivum* Kolibri/Ibis), which were used to produce the pasta and bread, respectively. In addition, we assessed how much free A and X is formed as a result of the production process of pasta and bread. For this analysis bread and pasta from another study (20) were used due to lack of products in the current study. These products were made from white wheat flour and wheat bran (7 %) both derived from *T. aestivum* var Capo. It was assumed that formation of A and X during pasta and bread making is not influenced by the origin of the flour or wheat bran. Pasta was analyzed raw and after being cooked for 10 min. Samples were homogenized by grinding. In all samples, the water content was also measured by using a moisture meter to determine the concentrations of bound and free A and X per dry weight unit.

For the analysis of free A and X, 5 g of ground product was extracted with 50 ml of MilliQ water for 16 h. For the analysis of total A and X, 100 mg of ground product was homogenized and extracted with 30 ml of 2 M sulphuric acid solution in MilliQ water for 1 h at 100° C. The extracts were diluted 10 times and injected into the High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD, Dionex) analysis system.

Analyses were performed using CarboPac PA1 (50 x 4 mm, Dionex) guard column and a CarboPac PA1 (250 x 4 mm, Dionex) analysis column. The temperature of the column was maintained at 22° C. 50 µl of (diluted) extract was injected, and the separation was performed with a flow of 1 mL/min with the use of a gradient as follows: 0.0 – 19.0 min NaOH 15 mM; 19.1 –24.0 min Acetate 100 mM / NaOH 100 mM; 24.0 –32.0 min NaOH 100 mM; 32.0 – 40.0 min NaOH 15 mM.

All eluents were degassed before use by flushing helium; subsequently they were kept under a constant helium pressure (eluent degassing module, Dionex). Signals were integrated and interpreted by means of chromatography software (PeakNet 5.1 Dionex). Compounds were detected using pulsed amperometric detection (PAD, Dionex).

Quantification was performed by using a 1-point calibration with an external standard solution, which was analyzed every 6 samples. All samples were analyzed in duplicate, and the averaged concentrations reported. The concentrations of bound A and X were calculated by subtracting the measured concentrations of free (unbound) A or X from the measured concentrations of total A or X.

Statistical analyses and data evaluation

The data sets were analyzed using a two-way ANOVA on time, treatment and its interaction. In this mixed model time, treatment and its interaction were used as fixed factors, subject was used as random factor. Diagnostics were used to check if the ANOVA assumptions were valid. In case the ANOVA assumptions were not met the corresponding metabolite was LOG transformed and the ANOVA model was repeated on LOG transformed data. In case the ANOVA assumptions were

still not met, the data were checked for the presence of statistical outliers. If present, these were removed and the whole procedure was repeated. A sample was considered as a statistical outlier and removed if the residual was > 3 x √mse. False Discovery Rate adjustment was used to control the false discovery rate and hence to correct for multiple testing. In all statistical tests performed, the null hypothesis (no effect) was rejected at the 0.05 level of probability. In this study we evaluated metabolites with significant treatment x time interactions, metabolites with a significant treatment effect and metabolites with both a significant treatment and time effect. Only when no significant interaction between intervention and time was found, main intervention effects averaged over time were investigated. The statistical software package SAS (version 9.3; SAS Institute Inc, Cary, NC) was used for statistical analysis.

Partial least squares (PLS) (21) was applied on autoscaled data in order to correlate GC-MS time resolved data (delta values) to several time resolved metabolic effects of interest (delta values). The endpoints used were insulin response, RaE, GCR, GIP and glucagon as published in Eelderink et al. (16). The delta values were defined as 'value at time point of interest minus value at baseline'. In the PLS procedure the validity of the model was investigated using 10-fold double cross validation (DCV) (21). Variable selection was applied during the modeling process using the jackknife approach (22). In this way, the importance of the metabolite for the model was evaluated, also referred to as the stability of metabolites. Using the RSD values, which were calculated during the variable selection procedure, instable metabolites were removed. By ordering the statistical importance of the stable and relevant metabolites by regression factors metabolites that contribute the most to a specific phenotype were identified. PLS models with R^2 mean >0.7 after 10-fold DCV were considered to be good models. Metabolites with a higher or equal to 0.1 absolute regression value were considered important contributing factors for the good PLS models. Ingenuity Pathway Analysis (Ingenuity System, Redwood City, CA, USA) was used to evaluate the differences in postprandial metabolism.

RESULTS

Plasma metabolites

The postprandial response of a total of 134 plasma metabolites with known identity (**Supplementary Table 1**) was quantified before (t= -5), and 30, 60, 90, 120 and 180 min after either bread or pasta consumption. ANOVA revealed a significant treatment x time effect for 9 (**Figure 1**), a significant treatment effect for 7 (**Table 1, Supplementary Figure 1**) and a significant treatment and time effect for 15 plasma metabolites (Table 1, **Supplementary Figure 2**). To further unravel plasma metabolites that could explain differences in postprandial metabolism, PLS modeling was applied to relate the plasma metabolome to postprandial insulin, GIP, RaE and GCR as parameters earlier reported to have different postprandial responses after pasta vs. bread consumption (16). For insulin and GCR good PLS models were found and the most contributing plasma metabolites to these PLS models are summarized in **Table 2**. PLS identified an additional 11 plasma metabolites with

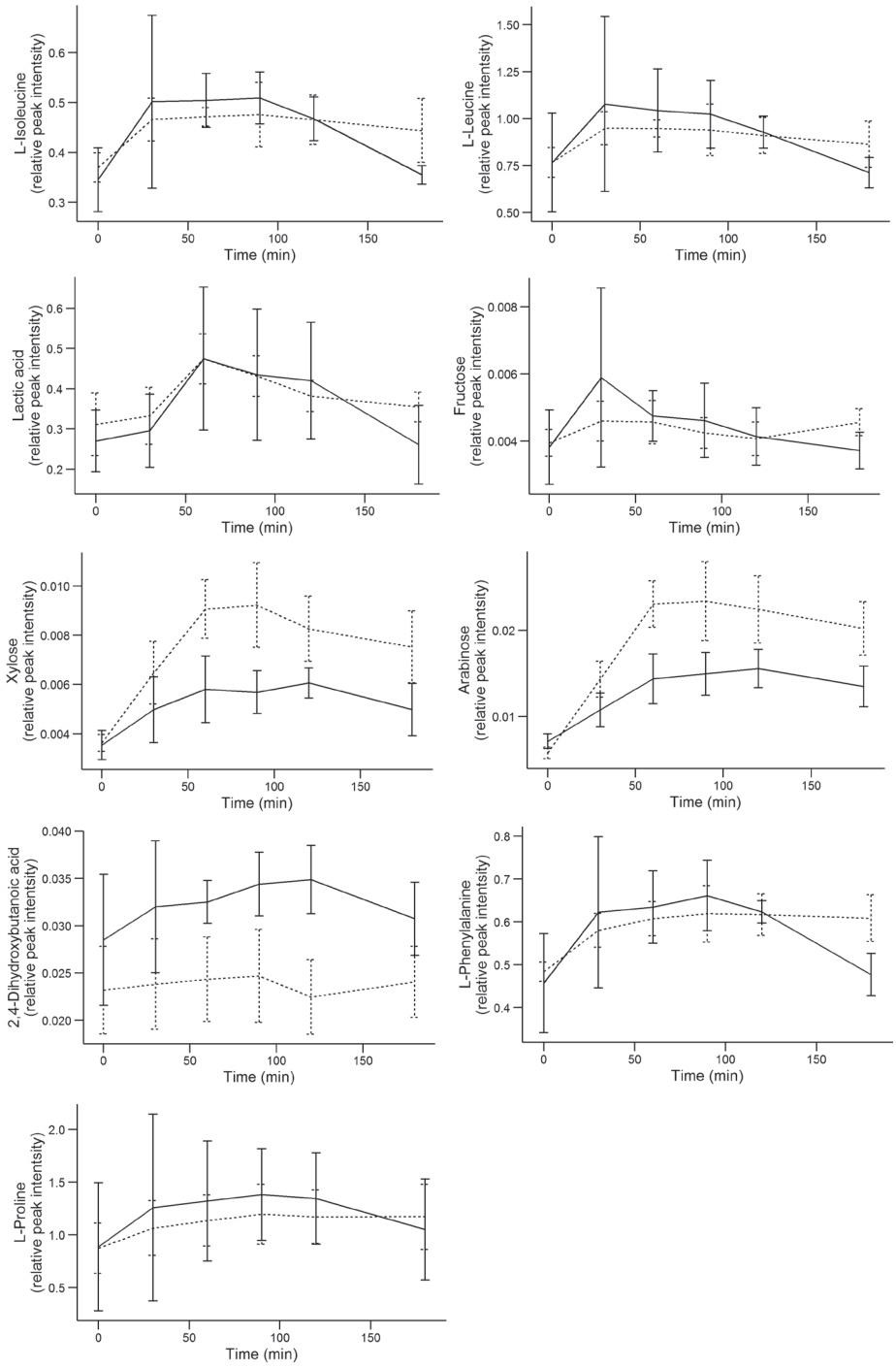

Figure 1. *Plasma metabolites with a significant treatment x time effect in nine healthy male volunteers participating in a crossover study consuming either wheat bread (black line) or pasta (dotted line) with postprandial sampling up to 3 h after consumption. Values are means ±SEM.*

Table 1. *Metabolites with a significant treatment, treatment and time or treatment x time effect in nine healthy male volunteers participating in a crossover study consuming either wheat bread or pasta with postprandial sampling up to 3 h after consumption*[1].

Variable	Treatment effect (*P* – value)	Time effect (*P* – value)	Treatment x time effect (*P* – value)	Response
L-Isoleucine			0.001	a>b[2]
L-Leucine			0.029	a>b[2]
Lactic acid			0.027	a>b[2]
Fructose			0.025	a>b[2]
Xylose			<0.001	b>a[2]
Arabinose			<0.001	b>a[2]
2,4-Dihydroxybutanoic acid			0.009	a>b[2]
L-Phenylalanine			<0.001	a>b[2]
L-Proline			<0.001	a>b[2]
1,2-Diglyceride (Mw 618)	0.030			a>b[3]
1-Methylhistidine	0.002			a>b[3]
Urea	0.001			a>b[3]
Sitosterol	0.021			a>b[3]
Glyceric acid	0.025			a>b[3]
Phosphate	0.006			a>b[3]
Fumaric acid	0.005			a>b[3]
Fatty acid 14:0	0.044	<0.001		a>b[4]
Fatty acid 17:0	0.026	<0.001		a>b[4]
Glycerol	0.035	<0.001		a>b[4]
Beta-Alanine	0.005	<0.001		b>a[4]
L-Valine	0.001	<0.001		a>b[4]
L-Tryptophan	0.001	0.007		a>b[4]
L-Tyrosine	0.003	<0.001		a>b[4]
Inositol	0.039	0.001		a>b[4]
Succinic acid	0.003	0.010		a>b[4]
Citric acid	0.012	<0.001		a>b[4]
L-Ornithine	0.020	<0.001		a>b[4]
Mannose	<0.001	<0.001		b>a[4]
2-Hydroxypiperidine	0.023	0.003		a>b[4]
Iminodiacetic acid	0.017	0.026		a>b[4]
Ribulose or Xylulose	<0.001	<0.001		a>b[4]

[1]a = bread; b= pasta; [2] postprandial responses are shown in Figure 1; [3]postprandial responses are shown in OSM Figure 1; [4]postprandial responses are shown in OSM Figure 2

a differential postprandial response in 9 healthy men who consumed pasta as compared to bread. This makes a total of 42 out of 134 plasma metabolites to have a different postprandial response. These 42 plasma metabolites covered different biological processes related to energy metabolism including glycolysis, citric acid cycle and urea cycle intermediates, most amino acids, medium chain fatty acids and glycerol, glucose and other monosaccharides such as fructose, ribose, xylulose or ribulose. These energy metabolism related plasma metabolites showed significant lower plasma

concentrations after consumption of pasta as compared to consumption of bread, indicating a lower energy generation through the oxidation of acetate derived from carbohydrates, fats and proteins into carbon dioxide and energy in the form of adenosine triphosphate (**Figures 2 and 3**). PLS regression on insulin indicates that insulin is an important driver for the difference in the metabolic response between pasta and bread, including GCR.

Next to reduced plasma concentrations of energy metabolism related metabolites including protein, lipid and carbohydrate metabolism, remarkable higher concentrations of A and X were found after consumption of pasta (Figure 1), which are derivatives from AX. Interestingly, the response of A and X is opposite as compared to the response of most metabolites. Furthermore, A and X were the metabolites which were the most significantly changed (*P*-interaction <0.0001 and 0.00035 respectively) (Figure 1).

Table 2. *Partial least square models that could be well predicted based on the metabolite profiling data from nine healthy male volunteers participating in a crossover study consuming either wheat bread or pasta with postprandial sampling up to 3 h after consumption*[1].

PLS model (mean-R^2)	Variable	Regression value
Insulin (0.721)	Glucose	0.232
	Lactic acid*	0.220
	Myo-inositol	-0.164
	L-Leucine*	0.158
	Beta-Alanine*	-0.156
	L-Ornithine*	-0.136
	L-Isoleucine*	0.122
	4-hydroxyglutamate semialdehyde	-0.122
	1,5-Anhydro-D-Glucitol	-0.110
	L-Tryptophan*	0.109
GCR (0.756)	Mannose*	-0.233
	Lactic acid*	0.182
	Pseudo uridine	-0.178
	Glucose	-0.160
	Alanine	0.152
	Fatty acid 10:0 *	-0.151
	L-Glutamine	0.150
	Vitamin E	-0.141
	L-Proline*	0.140
	L-4-Hydroxyproline	0.140
	isocitric acid	0.117
	Ribulose- or Xylulose*	0.109
	Citric acid*	0.103
	Ribose	0.103

[1]*GCR, glucose clearance rate; PLS, Partial least square*

**Metabolites with a significant treatment, treatment and time or treatment x time effect from Table 1*

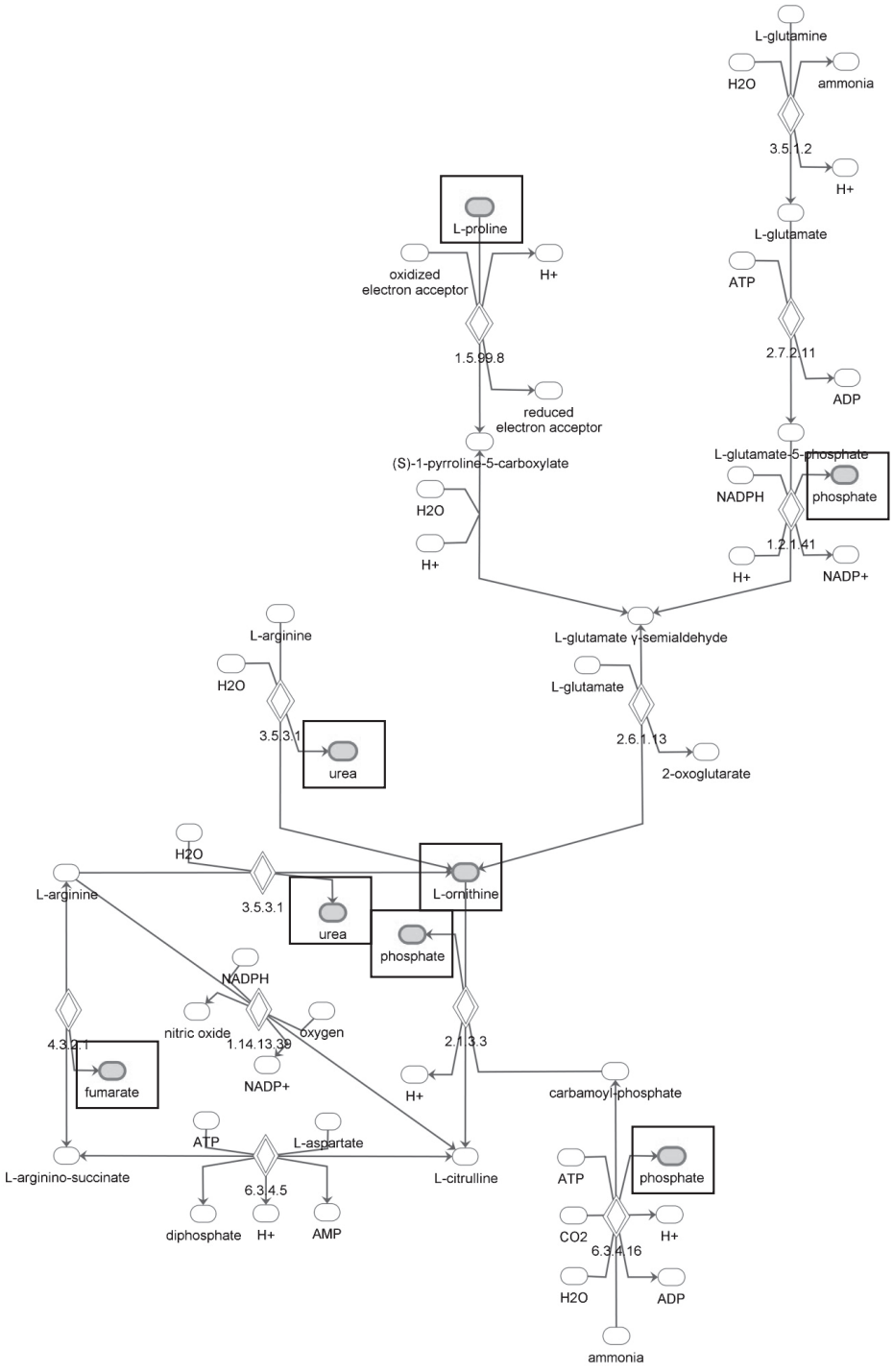

Figure 2. *Scheme of amino acid metabolism and urea cycle with metabolites indicated (box) that had lower levels in nine healthy male volunteers after consumption of wheat pasta as compared to bread.*

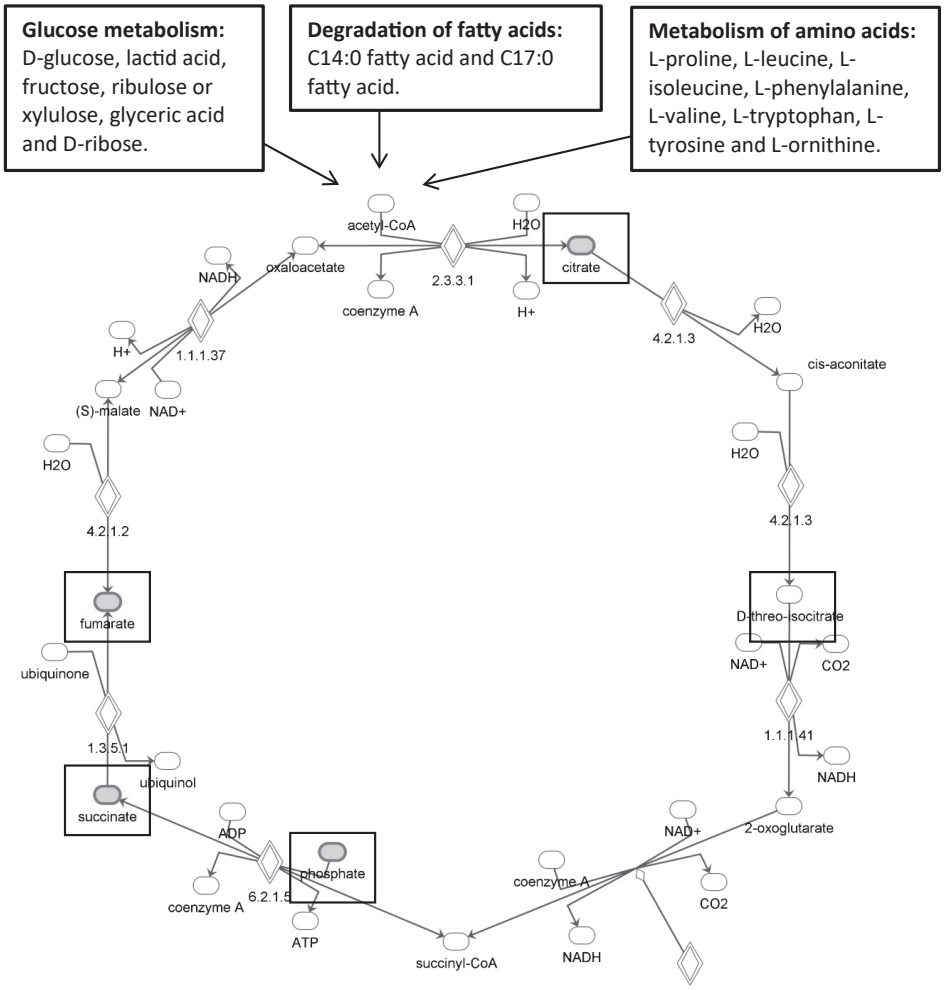

Figure 3. *Scheme of Citric Acid Cycle with metabolites indicated (box) that had lower levels in nine healthy male volunteers after consumption of wheat pasta as compared to bread.*

In vitro analysis of flour, bran and starchy products

Both types of flour contained no free A and X and their total amount of AX was the same (**Table 3**), which is in the range of percentages described in literature: 1.7 – 2.9 % AX in refined wheat flours (23) and 0.6 – 3.0 % in Durum wheat flour (24). Wheat bran contained, as expected, a higher amount of AX than flour (Table 3). From these data we calculated that one portion bread contained 3.6 g and one portion pasta 3.4 g AX (1.4 g from endosperm each and 2.1 and 1.9 g from bran respectively). In bread and cooked pasta no free A or X was found and in raw pasta only a negligible amount of A (0.1 g/100 g wet weight). Thus, during the production process of pasta and bread no A and X is formed.

Table 3. *Concentration of arabinoxylan, arabinose and xylose in flour and wheat bran used for the test products of this study*[1]**.**

Item	Wheat flour *T. aestivum* Kolibri/Ibis	Wheat flour *T. durum* de Cecco	Wheat bran from English Biscuit Wheat
Water, g/100 g	14.3	13.8	12.9
Free A, g/100 g wet weight	0.0	0.0	0.4
Free X, g/100 g wet weight	0.0	0.0	0.2
Bound A, g/100 g wet weight	0.8	0.8	8.3
Bound X, g/100 g wet weight	1.2	1.2	14.8
AX, g/100 g wet weight	2.0	2.0	23.1
Bound A, g/100 g dry weight	0.9	0.9	9.5
Bound X, g/100 g dry weight	1.4	1.4	17.0
AX, g/100 g dry weight	2.3	2.3	26.5

[1]*A, Arabinose; AX, arabinoxylan; X, xylose*

DISCUSSION

We applied targeted metabolic profiling for in-depth analyses of the postprandial changes occurring after consumption of 2 fiber-rich starchy products (bread and pasta), which differed in the rate at which starch-derived glucose appeared in the systemic circulation. Despite differences in RaE (slower after pasta), the total blood glucose response was the same after consumption of both products, whereas the insulin response was lower after the consumption of pasta (16). Lower plasma concentrations of metabolites related to energy metabolism were found after pasta consumption than after bread consumption. These differences were as expected based on the lower insulin response after pasta, which also was confirmed by PLS regression. Our results can only partly be compared with those of other studies. Postprandial analyses of metabolites have been conducted either after glucose challenges (5-7), after the consumption of breads with different macronutrient composition (4) or after the consumption of wheat bread while comparing results of metabolic different subgroups (25). An oral glucose load or consumption of wheat bread resulted in increased glycolysis and decreased lipolysis, which in our study was shown by, for example, increased lactic acid and decreased glycerol and free fatty acid concentrations. After pasta consumption, these effects were either less pronounced (glycolysis) or were suppressed for a longer time after pasta intake (lipolysis). Several amino acids increased postprandially due to the digestion of protein from our starchy products, and the kinetics, especially of leucine and isoleucine, were similar to those after the ingestion of refined wheat breads in other studies (4, 25). In our study, amino acid concentrations showed less of an increase and an attenuated decrease after pasta consumption than after bread consumption. Because pasta can be expected to have a slightly higher protein content, a lesser increase after pasta consumption could be explained by the slower absorption of amino acids, parallel to slower absorption of starch-derived glucose (RaE). Slower clearance of amino acids corresponded to the lower insulin response after pasta consumption.

An unexpected finding was the postprandial increase in arabinose and xylose concentrations after consumption of the meals, which, to our knowledge, has not been described before. Only in one other study were both metabolites detected and decreased in response to a fiber-free meal challenge, which can be due to insulin-mediated uptake of these sugars into cells (3).

Arabinose and xylose are derived from arabinoxylan (AX), a hemicellulose, which is an important component of wheat bran and also present in the starchy endosperm of the wheat grain. Its main feature is a X backbone with A side chains but its specific composition varies dependent on the location in the grain (23). In wheat bran, AX is an important part (about 32 %) of the non-starch polysaccharides (26) and wheat flour contains about 2.2 % AX (1.7 – 2.9 %) which is located in the cell walls of the endosperm (23).

Isolated AX from endosperm added to a starchy meal has been shown to lower postprandial glycemic response (27, 28) and for this function even an European Food Safety Authority (EFSA) health claim was approved (29). The mechanism by which this effect is exerted is considered to be well known and ascribed to increased viscosity of the meal bolus after consumption of soluble fiber interfering with absorption of glucose in the small intestine (29). However, it should be noted that other mechanisms could also play a role, as certain types of dietary fiber are implicated with the potency to inhibit starch digestion directly (30, 31). Intriguingly, in our study the RaE after pasta is significantly lower than after bread and coincides with higher plasma concentrations of A and X.

The question now arises how the early rise in plasma A and X can be explained, as fibers are not considered to be broken down in the small intestine. One possibility is that free A and X are already present in the products. However, analyses of pasta and bread showed that there is no free A and X formed. Thus, this apparently leaves only the possibility that AX is degraded in one way or another on its passage through the gastrointestinal tract. One study could be found that demonstrated that A in hemicellulose isolated from corn hull can be released under acidic conditions (32). After 3 h at 37° C and pH 1, 10% of A was released, but only 0.3 % of X. An increase of the pH to 1.5 and 2 decreased the release of A to 2.9 and 1% respectively with apparently no release of X. When we assume that wheat AX behaves similar, the probability of gastric release (pH 1 - 3) of A and X is not very high. Furthermore, the nearly constant postprandial release of both A and X from 60 – 180 min suggests another mode of action.

Another possibility is that A and X are released due to enzymatic degradation of AX by the microbiota in the gut. Degradation of AX by colonic microbiota does not seem feasible in view of the early time points at which the release of A and X occurred; it rather suggests activity of the small intestinal microbiota. It is known that the small intestine harbors a range of facultative aerobe and anaerobe microbial/bacterial groups although it has a lower species diversity and density than the large intestine (33, 34). Functional analysis of the small intestinal microbiota revealed that it not only uses and converts simple carbohydrates (35), but can also degrade more complex compounds like inulin and other oligosaccharides (36). More specifically, glycoside hydrolase (GH) families 10 and 43 were identified, which also comprise the enzymes needed to degrade

AX, namely endo-1,4-ß-xylanase (xylanase, EC 3.2.1.8), ß-xylosidase (EC 3.2.1.37), and α-l-arabinofuranosidase (arabinofuranosidase, EC 3.2.1.55) (37). These GH families were associated with the *Bacteroidales* group (36), which was also identified to be present in the human small intestine in other studies (33, 35, 38, 39). In addition, *Firmicutes* (especially *Bacilli*) and *Actinobacteria* (e.g. bifidobacteria) have been shown to be present even in the duodenal microbiota (40-42) members of which are able to utilize AX (36, 43).

Thus, the small intestinal microbiota has the potential to degrade AX. An important characteristic of dietary fiber that facilitates microbial fermentation is its water solubility (44). Unprocessed AX, however, are largely not water soluble, because 70 – 80 % of AX in endosperm (45) and ~94 % of AX in bran (26) is water-unextractable (WU). Processing, however, might change these characteristics, which will be further discussed below. During bacterial degradation, viscosity is expected to increase, because it has been shown that the activity of endoxylanases renders WU-AX more water soluble and viscous (45). Solubilized WU-AX fragments and water-extractable viscous AX are then further degraded to fragments of low molecular weight and subsequently viscosity again decreases (45).

Taken together, it seems that microbial degradation of AX in the small intestine is possible, and during this process the viscosity of the small intestinal contents may increase and contribute to slower digestion of starch by restricting diffusion of α-amylase towards starch and/or absorption of starch-derived glucose, reflected by the RaE in our study. In ileal digesta of pigs, soluble AX has been shown to be the most important factor that is responsible for differences in viscosity after diets with various AX containing breads (46). Increased viscosity coincided with low digestibility of starch after the rye aleurone bread despite a decrease of the molecular weight (25 %) of AX (46). Besides viscosity, it could be possible that A itself is involved in reducing the appearance of starch-derived glucose by inhibiting small intestinal maltase activity (47). However, results so far are contradictory (48, 49).

The remaining question is what causes the apparent difference in small intestinal AX degradation between our wheat pasta and bread? Because the amount of AX as well as the composition of both test meals were similar, the cause must lie in the differences in food product processing. Obvious differences in processing were that pasta dough underwent sheeting (pasta machine by Trattorina) after mixing (no drying) and cooking for 6.5 min in water, whereas bread dough after mixing was left to rise for 30 min and baked for 30 min at 240 °C. In addition, pasta was consumed directly after cooking and bread was frozen at – 20°C and thawed before consumption.

Information on how food-processing conditions influence AX solubility and molecular weight is only available for endosperm-derived AX (50, 51). However, in our bread and pasta also bran AX was present and constituted the larger part of AX (ca. 2 g vs 1.4 g from endosperm). Bran AX might behave differently due to specific features such as for example presence of high amounts of ferulic acid and lignin which might influence its water solubility through interaction with AX (23, 52). One study in pigs (46) showed that after consumption of whole meal wheat bread (which contains AX from endosperm and also from bran like in our bread) viscosity did not change and molecular weight of AX increased in ileal digesta, which is in line with what we would expect based on our

data. However, no data on the behavior of AX from whole meal pasta during small intestinal transit are available.

In conclusion, it seems feasible that arabinoxylans are degraded by the small intestinal microbiota. The higher solubility of AX in pasta may facilitate their degradation and increase small intestinal viscosity. This also suggests that wheat bran, depending on food processing, can, by means of increased viscosity, interfere with macronutrient absorption or digestion in the small intestine and thereby influence postprandial glucose and insulin responses. The effect of processing on bran AX therefore warrants further exploration.

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AJP, SW, CE, RJV, IB, MGP designed the research; LvS conducted the research; JHS, SB analyzed data and performed statistical analysis; AJP, SW, CE, IB, RJV, MGP wrote the paper; MGP had primary responsibility for final content. All authors read and approved the final manuscript.

REFERENCES

1. Barton S, Navarro SL, Buas MF, Schwarz Y, Gu H, Djukovic D, Raftery D, Kratz M, Neuhouser ML, Lampe JW. Targeted plasma metabolome response to variations in dietary glycemic load in a randomized, controlled, crossover feeding trial in healthy adults. Food Funct 2015 Sep;6:2949-56.
2. Campbell C, Grapov D, Fiehn O, Chandler CJ, Burnett DJ, Souza EC, Casazza GA, Gustafson MB, Keim NL, Newman JW, et al. Improved metabolic health alters host metabolism in parallel with changes in systemic xeno-metabolites of gut origin. PLoS One 2014;9:e84260.
3. Pellis L, van Erk MJ, van Ommen B, Bakker GC, Hendriks HF, Cnubben NH, Kleemann R, van Someren EP, Bobeldijk I, Rubingh CM, et al. Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. Metabolomics 2012 Apr;8:347-59.
4. Moazzami AA, Shrestha A, Morrison DA, Poutanen K, Mykkanen H. Metabolomics reveals differences in postprandial responses to breads and fasting metabolic characteristics associated with postprandial insulin demand in postmenopausal women. J Nutr 2014 Jun;144:807-14.
5. Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, Carr SA, Thadhani R, Gerszten RE, Mootha VK. Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol 2008;4:214.
6. Wopereis S, Rubingh CM, van Erk MJ, Verheij ER, van VT, Cnubben NH, Smilde AK, van der Greef J, van Ommen B, Hendriks HF. Metabolic profiling of the response to an oral glucose tolerance test detects subtle metabolic changes. PLoS One 2009;4:e4525.
7. Zhao X, Peter A, Fritsche J, Elcnerova M, Fritsche A, Haring HU, Schleicher ED, Xu G, Lehmann R. Changes of the plasma metabolome during an oral glucose tolerance test: is there more than glucose to look at? Am J Physiol Endocrinol Metab 2009 Feb;296:E384-E393.
8. Cho SS, Qi L, Fahey GC Jr, Klurfeld DM. Consumption of cereal fiber, mixtures of whole grains and bran, and whole grains and risk reduction in type 2 diabetes, obesity, and cardiovascular disease. Am J Clin Nutr 2013 Aug;98:594-619.
9. Priebe MG, van Binsbergen JJ, de VR, Vonk RJ. Whole grain foods for the prevention of type 2 diabetes mellitus. Cochrane Database Syst Rev 2008;CD006061.
10. Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr 2004 Aug;80:348-56.
11. Schwingshackl L, Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutr Metab Cardiovasc Dis 2013 Aug;23:699-706.
12. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev 2010 Jun;23:65-134.
13. Gonzalez-Anton C, Lopez-Millan B, Rico MC, Sanchez-Rodriguez E, Ruiz-Lopez MD, Gil A, Mesa MD. An enriched, cereal-based bread affects appetite ratings and glycemic, insulinemic, and gastrointestinal hormone responses in healthy adults in a randomized, controlled trial. J Nutr 2015 Feb;145:231-8.
14. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 2002 May 8;287:2414-23.
15. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 2002 Jul;76:274S-80S.
16. Eelderink C, Schepers M, Preston T, Vonk RJ, Oudhuis L, Priebe MG. Slowly and rapidly digestible starchy foods can elicit a similar glycemic response because of differential tissue glucose uptake in healthy men. Am J Clin Nutr 2012 Nov;96:1017-24.
17. Eelderink C, Moerdijk-Poortvliet TC, Wang H, Schepers M, Preston T, Boer T, Vonk RJ, Schierbeek H, Priebe MG. The glycemic response does not reflect the *in vivo* starch digestibility of fiber-rich wheat products in

healthy men. J Nutr 2012 Feb;142:258-63.

18. Koek MM, van der Kloet FM, Kleemann R, Kooistra T, Verheij ER, Hankemeier T. Semi-automated non-target processing in GC x GC-MS metabolomics analysis: applicability for biomedical studies. Metabolomics 2011 Mar;7:1-14.
19. van der Kloet FM, Bobeldijk I, Verheij ER, Jellema RH. Analytical error reduction using single point calibration for accurate and precise metabolomic phenotyping. J Proteome Res 2009 Nov;8:5132-41.
20. Eelderink C, Noort MW, Sozer N, Koehorst M, Holst JJ, Deacon CF, Rehfeld JF, Poutanen K, Vonk RJ, Oudhuis L, et al. The structure of wheat bread influences the postprandial metabolic response in healthy men. Food Funct 2015 Oct;6:3236-48.
21. Smit S, van Breemen MJ, Hoefsloot HC, Smilde AK, Aerts JM, de Koster CG. Assessing the statistical validity of proteomics based biomarkers. Anal Chim Acta 2007 Jun 5;592:210-7.
22. Bijlsma S, Bobeldijk I, Verheij ER, Ramaker R, Kochhar S, Macdonald IA, van Ommen B, Smilde AK. Large-scale human metabolomics studies: a strategy for data (pre-) processing and validation. Anal Chem 2006 Jan 15;78:567-74.
23. Saulnier L, Sado P, Branlard G, Charmet G, Guillon F. Wheat arabinoxylans: Exploiting variation in amount and composition to develop enhanced varieties. Journal of Cereal Science 2007;46:261-81.
24. Lempereur I, Rouau X, Abecassis J. Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (Triticum durum L.) grain and its milling fractions. Journal of Cereal Science 1997;25:103-10.
25. Shrestha A, Mullner E, Poutanen K, Mykkanen H, Moazzami AA. Metabolic changes in serum metabolome in response to a meal. Eur J Nutr 2015 Dec 10.
26. Maes C, Delcour JA. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. Journal of Cereal Science 2002;35:315-26.
27. Hartvigsen ML, Laerke HN, Overgaard A, Holst JJ, Bach Knudsen KE, Hermansen K. Postprandial effects of test meals including concentrated arabinoxylan and whole grain rye in subjects with the metabolic syndrome: a randomised study. Eur J Clin Nutr 2014 May;68:567-74.
28. Lu ZX, Walker KZ, Muir JG, Mascara T, O'Dea K. Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. Am J Clin Nutr 2000 May;71:1123-8.
29. EFSA Panel on Dietetic Products NaAN. Scientific Opinion on the substantiation of health claims related to arabinoxylan produced from wheat endosperm and reduction of post-prandial glycaemic responses (ID 830) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9:2205.
30. Dhital S, Gidley MJ, Warren FJ. Inhibition of amylase activity by cellulose: Kinetic analysis and nutritional implications. Carbohydrate Polymers 2015;123:305-12.
31. Slaughter SL, Ellis PR, Jackson EC, Butterworth PJ. The effect of guar galactomannan and water availability during hydrothermal processing on the hydrolysis of starch catalysed by pancreatic alpha-amylase. Biochim Biophys Acta 2002 May 10;1571:55-63.
32. Zhang P, Zhang Q, Whistler R. L-arabinose release from arabinoxylan and arabinogalactan under potential gastric acidities. CEREAL CHEMISTRY 2003 Jun;80:252-4.
33. Booijink CC, El-Aidy S, Rajilic-Stojanovic M, Heilig HG, Troost FJ, Smidt H, Kleerebezem M, de Vos WM, Zoetendal EG. High temporal and inter-individual variation detected in the human ileal microbiota. Environ Microbiol 2010 Dec;12:3213-27.
34. Cummings JH, Gibson RN, Macfarlane GT. Quantitative estimates of fermentation in the hind gut of man. Acta Vet Scand 1998;Suppl. 86:76-82.
35. Zoetendal EG, Raes J, van den Bogert B, Arumugam M, Booijink CC, Troost FJ, Bork P, Wels M, de Vos WM, Kleerebezem M. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. ISME J 2012 Jul;6:1415-26.

36. Cecchini DA, Laville E, Laguerre S, Robe P, Leclerc M, Dore J, Henrissat B, Remaud-Simeon M, Monsan P, Potocki-Veronese G. Functional metagenomics reveals novel pathways of prebiotic breakdown by human gut bacteria. PLoS One 2013;8:e72766.

37. Rasmussen L, Xu C, Sorensen J, Nielsena M, Meyer A. Enzyme kinetics and identification of the rate-limiting step of enzymatic arabinoxylan degradation. BIOCHEMICAL ENGINEERING JOURNAL 2012;69:8-16.

38. Hayashi H, Takahashi R, Nishi T, Sakamoto M, Benno Y. Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. J Med Microbiol 2005 Nov;54:1093-101.

39. Wang X, Heazlewood SP, Krause DO, Florin TH. Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. J Appl Microbiol 2003;95:508-20.

40. Angelakis E, Armougom F, Carriere F, Bachar D, Laugier R, Lagier JC, Robert C, Michelle C, Henrissat B, Raoult D. A Metagenomic Investigation of the Duodenal Microbiota Reveals Links with Obesity. PLoS One 2015;10:e0137784.

41. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalances in faecal and duodenal Bifidobacterium species composition in active and non-active coeliac disease. BMC Microbiol 2008;8:232.

42. Nistal E, Caminero A, Herran AR, Perez-Andres J, Vivas S, Ruiz de Morales JM, Saenz de Miera LE, Casqueiro J. Study of duodenal bacterial communities by 16S rRNA gene analysis in adults with active celiac disease vs non-celiac disease controls. J Appl Microbiol 2016 Jun;120:1691-700.

43. Lombard V, Golaconda RH, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 2014 Jan;42:D490-D495.

44. Guillon F, Champ M. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Research International 2000;33:233-45.

45. Courtin CM, Delcour JA. Arabinoxylans and Endoxylanases in Wheat Flour Bread-making. Journal of Cereal Science 2002;35:225-43.

46. Le Gall M, Eybye KL, Knudsen KEB. Molecular weight changes of arabinoxylans of wheat and rye incurred by the digestion processes in the upper gastrointestinal tract of pigs. Livestock Science 2010;134:72-5.

47. Jurgonski A, Krotkiewski M, Juskiewicz J, Billing-Marczak K. Suppression of postprandial glycaemia by L-Arabinose in rats is more associated with starch than sucrose ingestion - short report. Pol J Food Nutr Sci 2016;65:57-60.

48. Preuss HG, Echard B, Bagchi D, Stohs S. Inhibition by natural dietary substances of gastrointestinal absorption of starch and sucrose in rats and pigs: 1. Acute studies. Int J Med Sci 2007;4:196-202.

49. Seri K, Sanai K, Matsuo N, Kawakubo K, Xue C, Inoue S. L-arabinose selectively inhibits intestinal sucrase in an uncompetitive manner and suppresses glycemic response after sucrose ingestion in animals. Metabolism 1996 Nov;45:1368-74.

50. Ingelbrecht JA, Verwimp T, Grobet PJ, Delcour JA. Behavior of Triticum durum Desf. arabinoxylans and arabinogalactan peptides during industrial pasta processing. J Agric Food Chem 2001 Apr;49:1783-9.

51. Cleemput G, Booij C, Hessing M, Gruppen H, Delcour JA. Solubilisation and Changes in Molecular Weight Distribution of Arabinoxylans and Protein in Wheat Flours During Bread-Making, and the Effects of Endogenous Arabinoxylan Hydrolysing Enzymes. Journal of Cereal Science 1997;26:55-66.

52. Beaugrand J, Cronier D, Debeire P, Chabbert B. Arabinoxylan and hydroxycinnamate content of wheat bran in relation to endoxylanase susceptibility. Journal of Cereal Science 2004;40:223-30.

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Supplemental Table 1. *List of metabolites which were quantified in plasma samples of nine healthy men before (t= -5), and 30, 60, 90, 120 and 180 min after either bread or pasta consumption.*

#	Metabolite name	#	Metabolite name	#	Metabolite name
1	1,2,4-Trihydroxybenzene	46	Citric acid	92	Sphingomyelin (d18:1/18:0)
2	1,2-Diglyceride (Mw 618)	47	Creatinine	93	Sphingomyelin (d18:1/22:0)
3	1,2-Diglyceride (Mw 592)	48	D-Glucose	94	Sphingomyelin (d18:1/23:0)
4	1,2-Diglyceride (Mw 620)	49	D-Glutamic acid	95	Sphingomyelin (d18:1/24:0)
5	1,3-Diglyceride (Mw592)	50	D-Maltose	96	Sphingomyelin (d18:2/16:0)
6	1,3-Diglyceride (Mw620)	52	D-Ribose	97	Sphingomyelin (d18:2/18:0)
7	1,5-Anhydro-D-Glucitol	53	Erythronic acid	98	Sphingomyelin (d18:2/20:0)
8	1-Methylhistidine	54	Lysophosphatidic acid 16:0	99	Sphingomyelin (d18:2/24:0)
9	1-Monopalmitoylglycerol	55	Fructose	100	Lactic acid
10	1-Monostearoylglycerol	56	Glutamic acid internal amide	101	Meso-erythrytol
11	1-Monolineoylglycerol	57	Glyceric acid	102	Monomethylphosphate
12	1-Monooleoylglycerol	58	Glycerol	103	Myo-inositol
13	2,3,4-Trihydroxybutanoic acid	59	Glycine	104	Inositol cyclic phosphate
14	2,4-Dihydroxybutanoic acid	60	Hexadecanoic-methylester	105	N-carboxyl-alanine
15	2-Amino-1-butyric acid	61	HypoXanthine	106	N-carboxyproline
16	2-Hydroxybutanoic acid	62	Indole-3-propionic acid	107	N-carboxyvaline
17	2-Hydroxypentanoic acid	63	Inositol	108	N-methyl-4-hydroxyproline
18	2-Hydroxypiperidine	64	L-4-Hydroxyproline	109	Phosphate
19	2-monooleoylglycerol	65	L-Asparagine	110	Pipecolinic acid
20	2-monopalmitoylglycerol	66	L-Aspartic acid	111	Pseudo uridine
21	2-stearoylglycerol	67	L-Cysteine	112	Pyruvic acid
22	3-Amino-2-piperidinon	68	L-Glutamine	113	Quinic acid
23	3-Hydroxybutanoic acid	69	L-Histidine	114	Ribulose- or Xylulose
24	3-Methylhistidine	70	L-Isoleucine	115	S-methyl-L-cysteine
25	4-Methyl-2-oxovaleric acid	71	L-Leucine	116	Succinic acid
26	4-hydroxyglutamate semialdehyde	72	L-Lysine	117	Sucrose
27	4-oxoproline	73	L-Methionine	118	Threonine
28	Alanine	74	L-Ornithine	119	Ureum
29	Aminomalonic acid	75	L-Phenylalanine	120	Uric acid
30	Arabinose	76	L-Proline	121	Vitamin E
31	Arachidonic acid	77	L-Serine	122	Xylose
32	Beta-Alanine	78	L-Tryptophan	123	Fumaric acid
33	Fatty acid 10:0	79	L-Tyrosine	124	Glycolic acid
34	Fatty acid 12:0	80	L-Valine	125	Iminodiacetic acid
35	Fatty acid 14:0	81	Sphingomyelin (d16:1/16:0)	126	Isocitric acid
36	Fatty acid 16:0	82	Sphingomyelin (d16:1/18:0)	127	Malic acid
37	Fatty acid 16:1 (n-7)	83	Sphingomyelin (d16:1/20:0)	128	Methyluric acid isomer 1
38	Fatty acid 17:0	84	Sphingomyelin (d16:1/22:0)	129	Methyluric acid isomer 2
39	Fatty acid 18:0	85	Sphingomyelin (d16:1/24:1)	130	Oxalic acid
40	Fatty acid 18:1 (n-9)	86	Sphingomyelin (d17:1/16:0)	131	Phosphorylethanolamine
41	Fatty acid 18:2 (n-9 and n-6)	87	Sphingomyelin (d17:1/18:0)	132	Sitosterol
42	Methylester 18:2 (n-9 and n-6)	88	Sphingomyelin (d17:1/24:0)	133	Sn-Glycerol-3-Phosphate
43	Fatty acid 20:1 (n-9)	89	Sphingomyelin (d18:0/16:0)	134	Stearamide
44	Fatty acid 22:6 (n-3)	90	Sphingomyelin (d18:1/16:0)		
45	Cholesterol	91	Sphingomyelin (d18:1/17:0)		

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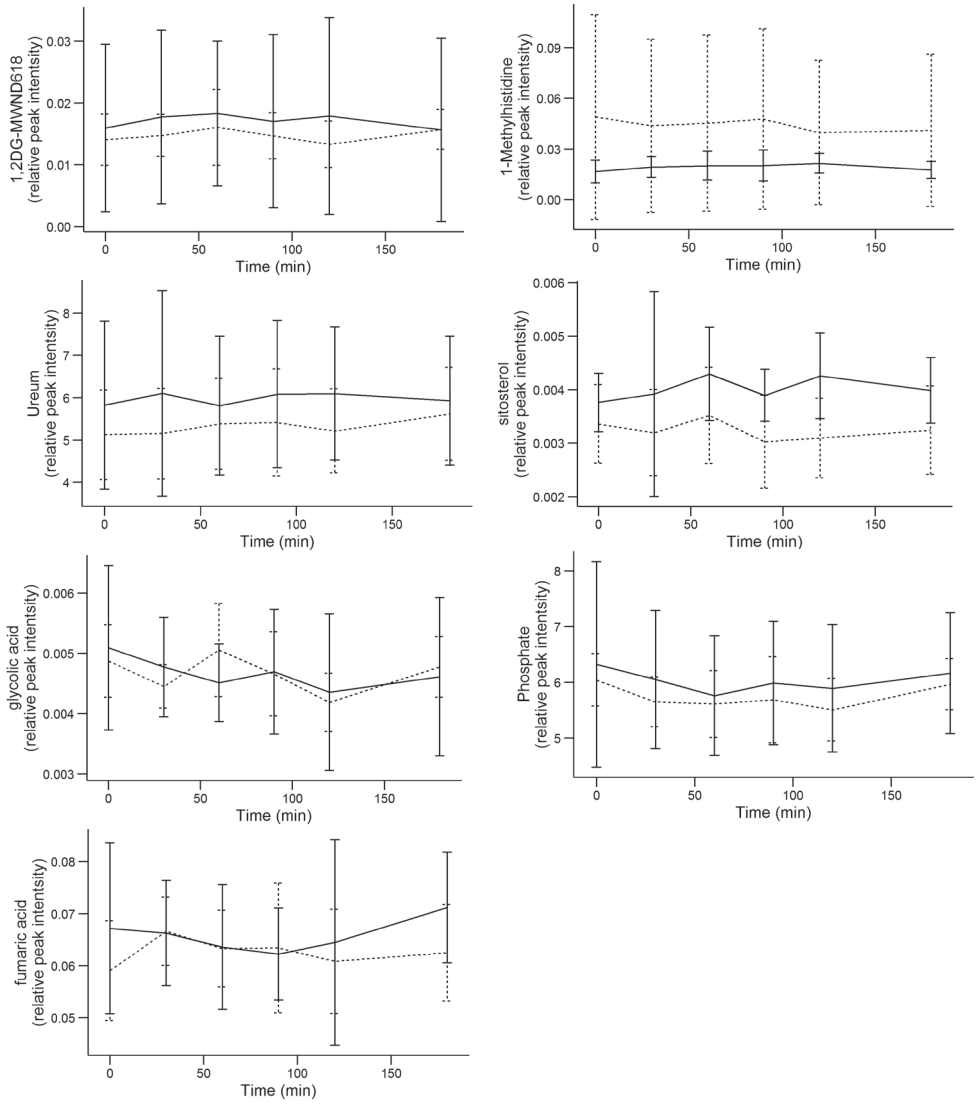
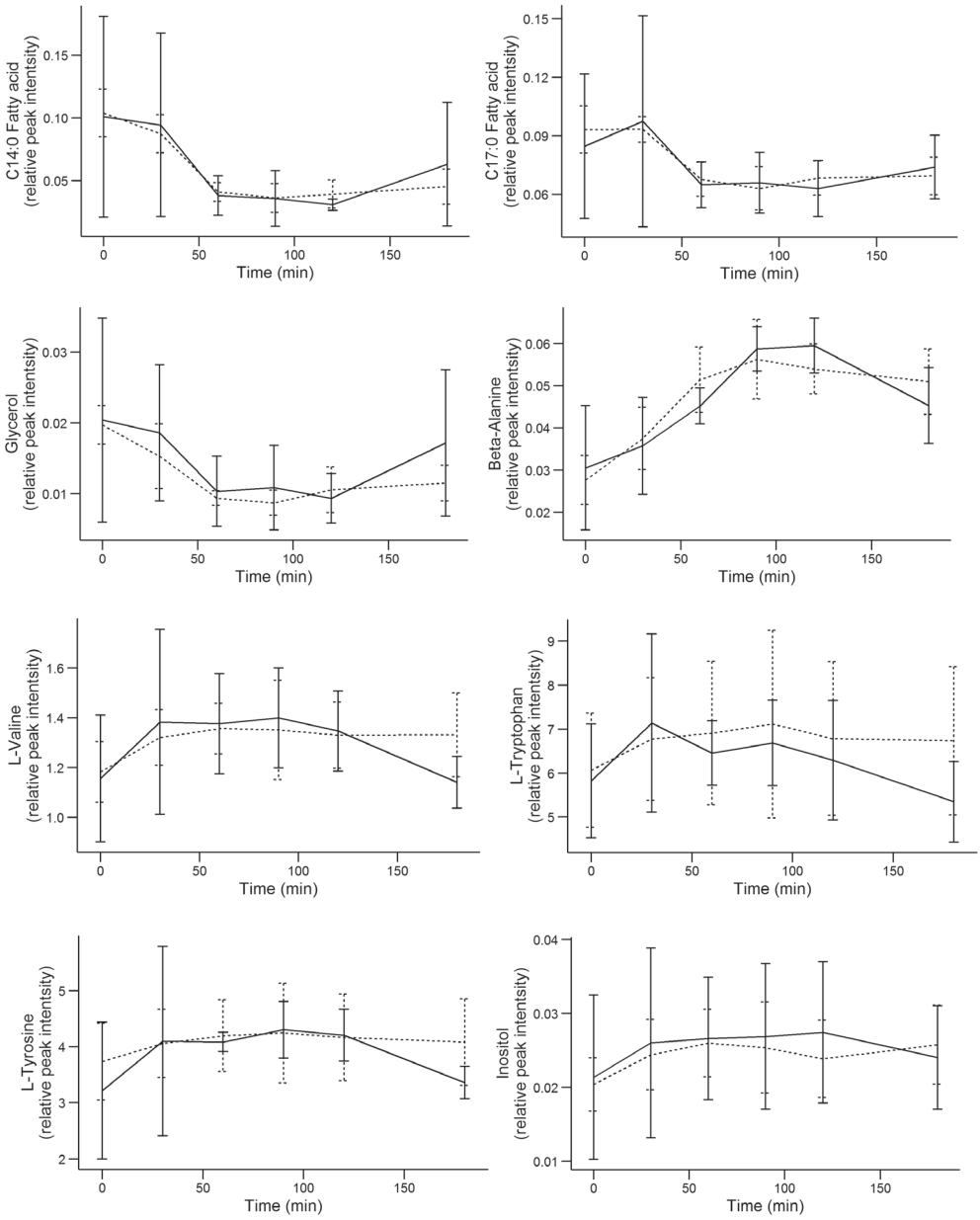
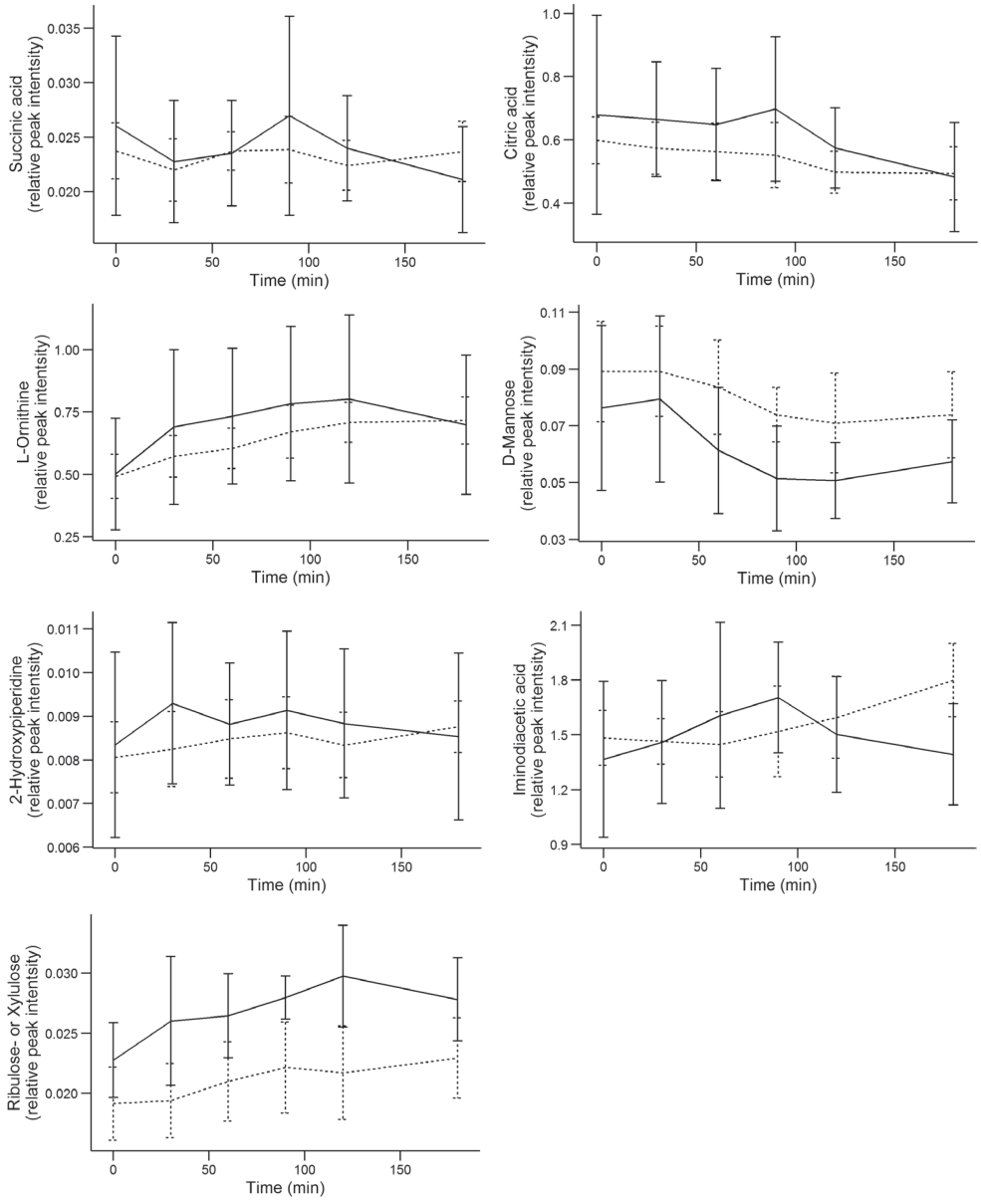


Supplemental Figure 1. *Plasma metabolites with a significant treatment effect in nine healthy male volunteers participating in a crossover study consuming either wheat bread (black line) or pasta (dotted line) with postprandial sampling up to 3 h after consumption. Values are means ± SEM.*



Supplemental Figure 2. *Plasma metabolites with a significant treatment and time effect in nine healthy male volunteers participating in a crossover study consuming either wheat bread (black line) or pasta (dotted line) with postprandial sampling up to 3 h after consumption. Values are means ± SEM.*



Supplemental Figure 2 (continued). *Plasma metabolites with a significant treatment and time effect in nine healthy male volunteers participating in a crossover study consuming either wheat bread (black line) or pasta (dotted line) with postprandial sampling up to 3 h after consumption. Values are means ± SEM.*

General discussion

PRELUDE

The development of Type 2 Diabetes Mellitus (T2DM) is highly lifestyle related, which asks for interventions with a focus on physical activity and/or diet aimed at the prevention of this disease or slowing its progression from pre-diabetes. Repeated high postprandial blood glucose concentrations, which may increase the risk for developing T2DM, is one factor that could be targeted by nutritional strategies. Starchy food products, such as bread, are widely consumed worldwide and their metabolic quality could be improved by decreasing their glycemic response, e.g. via starch digestibility. A better knowledge of the postprandial effects of starchy foods is crucial in order to understand their role in the development of (pre-)diabetes and to design more healthy starchy food products.

Therefore, the aim of this thesis was to increase our insight into the metabolic effects of starchy foods in healthy men by studying the postprandial response and the glucose kinetics of a variety of ^{13}C-labeled wheat products differing in composition and/or structure.

The following questions were addressed:

- What are the effects of product modification on the postprandial glucose response and underlying glucose kinetics?
- What are the effects of product modification on other postprandial metabolic factors like insulin, GIP, GLP-1, and bile acids?

Our findings (7.1 and 7.2) and their relevance for the prevention of T2DM (7.3) will be discussed in this final chapter.

7.1 THE EFFECTS OF PRODUCT MODIFICATION ON THE POSTPRANDIAL GLUCOSE RESPONSE AND UNDERLYING GLUCOSE KINETICS

To get a better understanding of the postprandial glucose response, it is crucial to calculate glucose kinetics after the consumption of the starchy food products under study. This provides us with more information about the different processes involved. Reduced postprandial glycemia is generally linked to slow starch digestion, so in our studies we attempted to decrease *in vivo* starch digestibility (RaE – Rate of appearance of Exogenous glucose) by modifying the composition or structure of wheat products. The main effects on glucose response and glucose kinetics will be discussed in this section.

Effects of modification of product composition

All our products were designed to contain 7 % dietary fiber, equivalent to the amount in whole grain products, by the use of wheat kernels or the addition of wheat bran. In the study described in Chapter 2, modifying wheat bread, we substituted normal wheat bran for purple wheat bran, resulting in

purple bran bread. Purple wheat contains the anthocyanin cyanidin-3-glucoside, which has shown inhibitory activity toward pancreatic α-amylase and α-glucosidase *in vitro* (1, 2). However, the addition of purple wheat bran in the wheat bread did not affect the RaE, from which we concluded that there was no inhibitory effect on the rate of starch digestion *in vivo*. The total glucose response was comparable, and even higher after purple bran bread 75 min postprandial, compared to the control bread (CB1). In another study in healthy subjects, the addition of polyphenol-rich berries to a starch-based food product had also no effect on the glycemic response (3). The lack of *in vivo* effect may be explained by the concentration of anthocyanins in the final food product, the form in which it was administered, or the influence of processing of the food product on the enzyme inhibiting capacity of cyanidin-3-glucoside.

The use of intact or broken grain kernels in bread formulations has also been reported to reduce the postprandial glucose response, mainly by limiting starch gelatinization and forming a physical barrier for α-amylase, and thereby reducing amylolysis (4). For instance, both the incorporation of 80 % pre-boiled whole wheat kernels (5, 6), as well as the replacement of flour (50 and 75 %) with cracked wheat (7), was previously reported to result in a reduction in GI. In the study described in Chapter 5, a kernel bread (KB) was prepared containing 85% broken wheat kernels. Unexpectedly, compared to a normal fiber-rich wheat bread (control bread, CB2), the postprandial glycemic response did not differ. Although this may still result from different glucose fluxes, the RaE reflecting '*in vivo* starch digestibility', as well as the glucose clearance rate (GCR) and the endogenous glucose production (EGP), was similar after KB compared to CB2 consumption. The broken kernels were clearly visible in the breads (Chapter 5, Fig 2), and extensive product characterization showed that due to the addition of broken kernels the average air cell wall thickness of KB was significantly larger compared to CB2. Moreover, KB had a higher density, and consequently a lower volume, which is a bread characteristic that could result in a lower glycemic response (8). However, the observed differences between CB2 and KB may have been insufficient to evoke a difference in glucose response or kinetics.

Effects of modification of product structure

As opposed to bread, pasta is a product often resulting in a low glycemic response, which is generally ascribed to the slow digestibility of starch (9). In the intervention study described in Chapter 2 and 3 we compared the postprandial response of a fiber-rich pasta meal (PA1) to a normal fiber-rich bread meal (CB1). Surprisingly, both products resulted in nearly the same postprandial glycemic response. Despite this remarkable similarity, there was a clear difference in glucose kinetics. RaE was slower after pasta, suggesting that there indeed was a difference in starch digestibility between both products. In addition, the GCR after pasta was slower, resulting in a prolonged presence of glucose in the circulation, which could explain why the glycemic response was similar after consumption of both products. It appeared that the GCR was closely 'matched' to the influx rate, influenced by insulin and GIP, as will be discussed later. With a high influx rate of glucose from the small intestine, such as

after consumption of control bread, the GCR is also high to prevent the glucose concentrations from rising too high. When the influx is rather slow (e.g. pasta, Chapter 3), the GCR is also slower, thereby counterbalancing the slow RaE, resulting in a similar 'high' glycemic response.

Based on our pasta results and reports in the literature (10-13), we hypothesized that a bread structure more comparable to the dense structure of our pasta, would induce a slower RaE via decreased starch digestibility, and may resemble the postprandial pasta (PA1) response (Chapter 4). Therefore, a bread with a compact structure, flat bread (FB), was designed. After FB consumption the RaE was indeed comparable to the low PA2 response and lower compared to CB2 in the first hour postprandial. Thereafter, the RaE was intermediate for FB compared to PA2 and CB2, possibly partly related to the fact that FB was less compact than PA due to arising air bubbles during processing. However, the compact structure of FB did seem to decrease the RaE to some extent, likely due to lower accessibility of starch degrading enzymes to their substrate. In addition, the decreased swelling and gelatinization of starch that we observed due to the short baking process may have contributed to the slower RaE after FB consumption. Corresponding to the findings with CB1 and PA1 regarding the similarity in glycemic response (Chapter 2 and 3), the total glucose response after consumption of CB2, FB and PA2 was also similar (iAUC), except that the peak value of glucose was somewhat lower for PA2 and FB compared to CB2 (Chapter 4).

It is clear from the described studies that in healthy subjects the glycemic response to different products is often very similar. As demonstrated in these studies, distinguishing between endogenous (EGP) and exogenous glucose (RaE) and taking into account the uptake rate of glucose into tissues (GCR) gives valuable additional information on how the glycemic response arises after consuming a certain starchy food product. The RaE itself, however, reflects a combination of several processes as well. As mentioned in Chapter 1, it can give an indication of the *in vivo* digestibility of starch. Consequently, the RaE comprises processes such as oral processing, gastric emptying, transit time and/or the uptake of starch-derived glucose (Chapter 1, Fig 2), which cannot be further distinguished. Therefore, often the exact reason for a slow or rapid RaE after consumption of a certain product is still an educated guess based on product characteristics and additional information from for instance *in vitro* and/or animal studies.

In our study comparing pasta and bread (Chapter 2 and 3) we observed a slow RaE after pasta consumption, which was expected to mainly result from slow digestibility of starch. However, metabolomics analyses of the plasma samples provided us with interesting information about the possible additional origin of the slow RaE of our pasta product (Chapter 6). Despite a slower influx of glucose into the systemic circulation, we found higher plasma concentrations of arabinose (A) and xylose (X) after pasta consumption. These sugar molecules originate from arabinoxylan (AX), a hemicellulose, which is an important component of wheat bran and also present in the starchy endosperm of the wheat grain (14). AX is known for its potency to increase viscosity (15) and decrease the postprandial glucose and insulin response (16). Notably, the higher concentrations of A and X after pasta consumption coincided with lower fluxes of other nutrients and several

metabolites, indicating a higher bioavailability of A and X from pasta. We speculated that AX, present in equal amounts in bread and pasta (mainly from wheat bran), was differently degraded by the *small* intestinal microbiota. The characteristics of the bran may be affected by processing of the food product (e.g. boiling), making AX from pasta more soluble and viscous, and thereby readily accessible for degradation, possibly further increasing viscosity of the meal bolus. These events could have induced either slower gastric emptying, decreased digestibility of starch (either by interference with starch degrading enzymes through viscosity (17) or by maltase-inhibition by arabinose (18)) and/or interference with uptake of glucose and other nutrients (17), resulting in a decreased RaE. These results could indicate that the slow influx of glucose after pasta consumption, next to possible other pasta related factors such as the dense product structure, was partly explained by the changes made to the characteristics of ingredients during the production process.

Summary - section 7.1

Taken together, in contrast to the addition of ingredients such as purple bran or broken wheat kernels, changing the structure of the products by different processing accounted for the largest differences in RaE. Modifying the product structure while keeping the product composition similar might nevertheless have changed the characteristics of the ingredients such as bran due to the different processing conditions, which may have had an additional effect on the postprandial response. The differences in RaE in our studies were not reflected by the glycemic response. In general, after consumption of products resulting in a slower RaE compared to a control bread, the glycemic response was similar due to the counterbalancing effect of the GCR. Thus, the glycemic response does not always reflect '*in vivo* starch digestibility' in healthy subjects, as the body is able to tightly regulate glucose homeostasis. The responses of the hormones that play an important role in these processes will be discussed in the next section.

7.2 THE EFFECTS OF PRODUCT MODIFICATION ON OTHER POSTPRANDIAL FACTORS

As we observed in our studies, the total glucose response and/or glucose kinetics do not always change due to product modifications, but this does not mean that other postprandial processes respond similarly as well. We measured several postprandial factors (including insulin, glucagon, GIP, GLP-1, CCK, BA) that may play a role in glucose metabolism and investigated their relationship to glucose kinetics. The most relevant observations will be discussed in this section.

GIP - regulation via glucose influx

The most obvious effect of product modification on GIP was a decreased response after consumption of products with a compact food structure, PA and FB, after which the RaE was lower as well. In all studies described in this thesis, RaE and GIP showed a strong correlation (mean correlation coefficient ~0.8), which is in accordance with a previous study from our group using corn products (19). Although RaE represents the rate of appearance of glucose in the systemic circulation, it is

also an indication of the rate of intestinal uptake of starch-derived glucose. The relation between intestinal glucose absorption and GIP release (dose-response) was also shown in several duodenal perfusion studies (20-22). This relationship can be explained by the importance of the intestinal sodium-glucose transporter-1 (SGLT-1) in GIP secretion. The transporter is responsible for the uptake of glucose into enterocytes, but is also expressed by K-cells, where it acts as an intestinal glucose sensor and triggers GIP secretion (23).

The relationship between RaE and GIP was even more emphasized when we made some adjustments to the method of calculating glucose kinetics. Previously a first step was smoothing of the raw data (OOPSEG, used in Chapter 2), resulting in less irregular shapes in the calculated variables (e.g. RaT, RaE, EGP, GCR). However, omitting this smoothing step (Chapter 3) and calculating glucose kinetics with the raw, unsmoothed data from each subject resulted in RaE curves that were almost identical to the GIP responses in most cases. The close resemblance of individual patterns of RaE and GIP was also visible from the average data. For instance, after consumption of CB2 a peak in RaE at 120 min was also clearly present in GIP plasma concentration at the same time point (Chapter 5, Fig 4a and 5a).

In Chapter 3 we suggested that GIP, in contrast to total blood glucose, is a good reflection of the RaE, and could give additional information about '*in vivo* starch digestibility'. This is for instance helpful and informative in studies that do not use stable isotopes and therefore are unable to calculate RaE directly. However, in Chapter 4, the *exact rate* of appearance of glucose after consumption of FB was not reflected by the GIP response over the whole postprandial period (Fig 4a and 5a). Here, GIP concentrations were almost identical after FB and PA2, but the RaE was slightly higher after FB consumption between t=45 and t=105 (ns). It might be that the initial glucose influx rate is the most important factor in determining the extent of the GIP response, as the RaE at 30 min was the same after FB and PA2 in this early postprandial phase. In support of this, the initial rate of delivery of readily absorbable glucose to the duodenum was found to be important in determining the height of the incretin response; an initial rapid infusion rate of glucose during 15 min and a subsequent slower infusion rate resulted in a high peak in plasma GIP and GLP-1 responses around 30 min, whereas the same amount of calories infused at a constant rate resulted in a low incretin response (24). Similar effects could be expected for starch-derived glucose from a source containing both rapidly and slowly digestible starch.

Taken together, postprandial GIP response patterns closely resembled the RaE, emphasizing that the GIP response is clearly dependent on the intestinal uptake rate of glucose. Rapid glucose uptake (and consequent glucose influx into the systemic circulation as visualized by RaE) would evoke a high GIP response, resulting in an upregulated insulin response, whereas slow intestinal uptake of glucose would result in a low GIP response. Thus, it is clear that when aiming to influence the GIP response by changing product characteristics, the (mainly initial) rate of intestinal uptake of (starch-derived) glucose is a crucial target for modification.

Insulin - discrepancy with glucose

In general, plasma glucose and insulin responses closely resemble each other after the consumption of starchy food products (25-27). It was clearly seen after intake of most of our products that, with the pattern of glucose and insulin being remarkably similar, the postprandial insulin response was largely determined by glucose concentrations, as sensed by the pancreatic β-cells. However, in Chapter 3 we found that the insulin response to pasta (PA1) was much lower as would be expected from the glycemic response. In addition, the insulin response was also clearly lower after FB compared to CB2 (Chapter 4) despite similar glucose responses, and was comparable to the low insulin response after PA2 consumption. The intensity of the insulin response can be greatly influenced by the incretins (28), and is therefore partly depending on the intestinal influx rate of glucose. After consumption of these products the RaE and GIP response were indeed lower compared to the control bread.

This discrepancy in glucose and insulin response was also described by others after intake of certain types of bread. For instance, several rye breads elicited a glycemic response comparable to that of white wheat bread, whereas the insulin and incretin responses were significantly lower (10, 11, 29). This response was low after endosperm rye bread, traditional whole-meal and high fiber rye bread (11) and was described as the 'Rye factor'. Similarly, however, in comparison with conventional breads the consumption of some differently processed wheat breads also resulted in a similar glucose response but a lower insulin (13) or GIP (12) response. The two products characterized by a low insulin response were a wheat bread fermented with mainly leaven from a starter culture composed of lactobacilli used to produce traditional sourdough bread, and a traditional baguette, for which less yeast (also 60% less yeast compared to the reference) and no ascorbic acid was used and a traditional (less intensive) mixing and kneading procedure was applied (13). The breads resulting in a low GIP response (and a tendency toward a lower insulin response) were made from the ancient Einkorn wheat, one using honey-salt leavening and the other using crushed whole grains (12). An Einkorn bread made in the same way as the conventional yeast leavened reference bread made from modern wheat did not show this decreased response, indicating the production process to be responsible.

These differences in bread preparation, all described as more traditional bread making, were suggested to have decreased the digestibility of starch, as indicated by a more rigid bread structure of the traditional baguette (13) or the lower content of rapidly available glucose *in vitro* (12). Lower hydrolysis indexes compared with white wheat bread were indications for a slower digestibility of starch in the rye products (10, 11). This was explained by a decreased accessibility of starch by degrading enzymes due to a different (microscopic) structure of rye breads (11). As these rye products were sourdough breads (combination of yeast and lactobacilli), the presence of certain produced organic acids (30) may play a role, as was a proposed explanation for the decreased insulin response to the previously mentioned bread fermented with leaven (13) whether or not via decreased gastric emptying (31).

A common characteristic of the described breads was their compact structure, which may have

resulted in a decreased intestinal glucose influx rate. Consumption of such products may result in larger food particles due to differences in chewing. The physical structure of food (texture, density, stiffness) determines the size of particles resulting from mastication in the mouth (32). Pasta was found to result in larger and denser particles, less mixed with saliva, compared to bread, likely resulting in decreased starch degradation. Particle sizes of several variations of rye and wheat breads were also compared after chewing and additional incubation with pepsin, which showed that the microstructure of the particles of rye breads (sourdough breads, also resulting in a low insulin response) was much less disintegrated compared to wheat breads (33). In turn, larger particle size of the food bolus can decrease gastric emptying, due to the slower disintegration rate (34).

In addition, viscosity of the meal bolus in the small intestine can play a role. Indications for this possible effect come from our metabolomics results comparing CB and PA (Chapter 6), where postprandial A and X concentrations in plasma were higher after pasta consumption, as discussed earlier. High concentrations of AX are also found in rye (products) (35). When isolated AX from the soluble fraction of wheat was added to wheat bread, similar results concerning a low insulin response were found compared to a ground rye bread, in catheterized pigs (36). This suggests that this component may play a significant role in the 'rye factor', and may be a relevant ingredient to consider when aiming at slower glucose influx and low postprandial GIP and insulin responses. Sourdough fermentation may further increase solubility of AX, as was found for whole meal wheat bread (37).

Thus the observed discrepancy in glucose and insulin response to these breads and pasta might be the result of a slow appearance of starch-derived glucose (RaE). This would result in a low GIP and insulin response, and thereby in a slow GCR and a 'high' glycemic response, as we first described in Chapter 3. This probably holds regardless of the reason for the slower RaE, which would need further study.

Insulin related effects on GCR and EGP

Insulin stimulates glucose uptake into insulin sensitive tissues via GLUT4 translocation to the membrane, and therefore a low insulin response would be expected to result in a slow GCR. This effect was indeed observed after consumption of CB1 and PA1 (Chapter 2 and 3) and in another previous study comparing breakfast cereals (38). Interestingly, the GCR after FB consumption was higher than expected based on the insulin response (Chapter 4). Although insulin was similarly low after both FB and PA2, the GCR was higher after FB consumption. In that way, the GCR after FB and CB2 was almost identical, despite the lower insulin response to FB as compared to CB2.

This relatively high glucose disposal after FB may be explained by either increased peripheral insulin sensitivity or due to insulin-independent glucose clearance. One factor involved may be GLP-1, which peaked after FB consumption but not after pasta intake. However, results on the effect of GLP-1 on insulin sensitivity are not convincing, and by using a hyperinsulinemic-euglycemic clamp to assess IS in healthy individuals during GLP-1 infusion, no effect was found (39). In Chapter

4 we described the possible involvement of BAs in increasing IS and glucose disposal. After FB consumption, the conjugated BA concentrations rose steeply (peak at t = 30 min), whereas no pronounced peak was observed after PA consumption. There are indications from studies in rodents (40-43) and humans (44) that BAs, via activation of their receptor FXR, could be involved in improving insulin sensitivity, normalization of hyperglycemia and/or increased glycogen storage. Similar beneficial effects for TGR5, such as improved insulin sensitivity, increased energy expenditure, and reduced inflammation, found in mainly pre-clinical studies were described in a recent review (45). This may indicate the potency of BAs in regulating insulin sensitivity and emphasizes the possible therapeutic potential of BAs in targeting metabolic diseases, although there is less known about acute effects of BAs and the translation of their effects to the human situation.

The relatively high GCR after FB consumption, together with a slower glucose influx, would be expected to result in a lower glucose response. However, the endogenous glucose flux could also be different between these meals. Indeed, EGP was slightly less suppressed after FB (ns) compared to CB2, likely due to low insulin concentrations. Although a direct effect of insulin is regarded as the primary determinant of EGP in humans (46), it must be noted that EGP does not always respond as expected based on insulin concentrations. The EGP after PA2 consumption, with a similar low insulin response compared to FB, was comparable to the EGP after CB2 consumption (high insulin response). In addition, an increased suppression of EGP despite low insulin concentrations has been observed after PA1 compared to CB1 in Chapter 3, and previously after bread consumption compared with glucose ingestion (47). Glucagon may play a role in this matter, as it was reported that the ratio between glucagon and insulin would mainly determine EGP (48). Our glucagon results were however inconsistent, and unable to explain our findings. It could also indicate that in these cases EGP was partly suppressed independent of insulin (49), or that hepatic insulin sensitivity influenced the extent of EGP suppression (50).

GLP-1 response independent from glucose kinetics

The modification of our products did also affect the GLP-1 response, most prominently in the study described in Chapter 5 comparing CB and KB. GLP-1 secretion often shows a bi-phasic response, referred to as early and late postprandial response, which may be explained by the presence of L-cells in both the duodenum and the colon (51). It was expected that the larger particles in KB would result in more starch(-derived glucose) reaching the more abundant distal L-cells, resulting in an increased late postprandial GLP-1 response. In a study with slowly digestible starch, a prolonged influx of glucose together with an extended GLP-1 response was observed (19). However, in our study the intestinal glucose uptake (RaE) after consumption of CB and KB was very similar over the 6 hour postprandial period. Still, we observed a large difference in the GLP-1 response, which was lower after KB consumption compared to CB.

GLP-1 secretion in the early postprandial phase seems to involve the SGLT-1 glucose transporter in L-cells, similar to GIP secretion from K-cells (23, 52). When knocking out or blocking this glucose

transporter in mice, the early GIP and GLP-1 responses (measured before t=15) to glucose infusion in the small intestine were reduced (23, 52). However, in a similar experiment (53), it was observed that this inadequate functioning of SGLT-1 resulted in higher concentration of glucose in the small intestine and colon for several hours after an OGTT, and resulted in an increased GLP-1 response compared to controls, whereas the results for GIP showed the opposite (53). This suggests that, except for the early response, the actual uptake of glucose via SGLT-1 is less crucial for stimulating GLP-1 release. In agreement, the correlations between the postprandial RaE and GLP-1 response (correlation coefficient ~0.5-0.7) after the different test meals were lower compared with correlations between RaE and GIP (mean correlation coefficient ~0.8) in our studies. Together, this indicates that the regulation of GLP-1 release was more complex and that besides the intestinal uptake of glucose, additional factors were involved in stimulating GLP-1 secretion from L-cells.

Because L-cells are densely distributed in the distal small intestine and colon, it might be that only the presence of still unabsorbed carbohydrates is important in stimulating GLP-1 in the late postprandial phase, for instance via other (glucose-)sensing mechanisms. The RaE indicated that after CB and KB consumption similar amounts of glucose were taken up in to the circulation. However, the products also contained around 7% fiber, which may have increased small intestinal or colonic fermentation. Short chain fatty acids (SCFAs), produced during fermentation of unabsorbed carbohydrates or fiber by the intestinal microbiota, are known to stimulate GLP-1 secretion as well (54), and were found to increase GLP-1 concentrations in the late postprandial phase (55, 56). Although fermentation would mainly occur after ~5 hours of consumption, an early increase in plasma ^{13}C-SCFA (mainly acetate) was found previously within 3 hours after barley consumption (57). Thus, their influence on GLP-1 after CB and KB intake could be possible. The higher GLP-1 response after CB would then result from the presence of finer wheat bran compared to the broken kernels in KB, as finely ground wheat bran was found more readily fermented and more effective in butyrate production as compared to coarse wheat bran (58).

In addition, from *in vitro* and animal data we know that BAs are also able to potentiate GLP-1 release from L-cells via activation of the BA receptor TGR5 on the cell membrane (59-62). In healthy humans, GLP-1 concentrations increased after jejunal infusion with taurocholic acid and glucose (63). In Chapter 4 and 5 we studied the postprandial response of bile acids; conjugated bile acids in plasma increased, whereas the unconjugated BAs decreased after bread consumption. Similar responses were observed after an OGTT (64, 65). After ingestion of a mixed meal, GLP-1 correlated with mainly glycine-conjugated BAs and total BAs (66). Corresponding with the latter study, the moderate correlations we found between postprandial GLP-1 and BA (correlation coefficient ~0.3-0.6) indicated that there may be a relationship between both responses. After KB consumption (Chapter 5), the total BA response tended to be lower compared to CB (from t = 90 min), which corresponded with the findings for GLP-1. In Chapter 4, we observed a sharp GLP-1 peak at t = 30 min after FB, which was very similar to that after CB2, but absent after PA2 consumption. Interestingly, when comparing GLP-1 responses with plasma BA concentrations after FB and PA2 we

observed similar response patterns, mainly with respect to the first postprandial hour. In accordance with the GLP-1 response, conjugated BA peaked after FB around 30 min postprandial, whereas PA2 consumption resulted only in a modest increase in BAs. It could thus be speculated that the high conjugated BA peak after FB had an additional effect on GLP-1 secretion, thereby explaining the initial high GLP-1 peak.

The reason for the 'difference' in plasma BA peaks after the breads is not evident. CCK, stimulating gall bladder contraction and thereby BA release, was only slightly different (ns) after the meals, and could not explain the pattern of BAs in plasma. However, for CCK less data points were available (the first postprandial time point was t=30). Although not apparent from the CCK response pattern we obtained, the time point around 15 min postprandial is likely to contain a peak in CCK response, as observed after increasing duodenal glucose loads (21) and a high-fat liquid meal (67). This could also explain the relatively low correspondence between CCK and BA patterns (Chapter 4 and 5). Although starch can result in a minor CCK response, the main stimulus for CCK is fat and protein (68), which was added in the same low amounts to our test meals. There are also indications that fiber affects the CCK response (69-71), and although fiber content was also similar in our products, fiber characteristics may have been different between products, which might evoke differences in CCK response. In this context, there is also a possibility that the fiber or bran in the food product is of influence on the BA (and GLP-1) concentrations directly. Reabsorption of BA normally happens in the small intestine as part of the enterohepatic circulation of BA. However, fiber was found to bind BAs to a certain extent depending on the type (72), thereby preventing BA reabsorption by sequestration (73), and affecting BA plasma concentrations as well. BA bound to fiber could also result in higher exposure of the L-cells to BAs in the distal small intestine and colon, thereby contributing to GLP-1 secretion (74, 75).

Because of the complex regulation of GLP-1 release, several factors could simultaneously play a role after consumption of starchy food products such as bread. GLP-1 analogues are successfully used in the treatment of T2DM. However, to understand postprandial GLP-1 stimulation and the possible role of BAs, more research is necessary. Their relevance in relation to the prevention of T2DM will be discussed later.

Summary - section 7.2

Taken together, several postprandial factors were affected by product modification. The responses of GIP and insulin were mainly determined by the RaE. The GLP-1 response was less clearly related to RaE, and the observed difference in response was probably evoked by other product characteristics or released factors (e.g. fiber, BA, SCFA).

Thus, it is clear from the results presented in this section that despite similar glucose responses, other postprandial factors may respond differently to the ingested food products. These additional postprandial (often glucose-regulating) responses should not be overlooked when studying the metabolic quality and health effects of starchy foods.

The postprandial responses and metabolic interactions described in 7.1 and 7.2 are summarized in a simplified scheme (**Figure 1**). The relevance and implications of the results will be discussed in the next section.

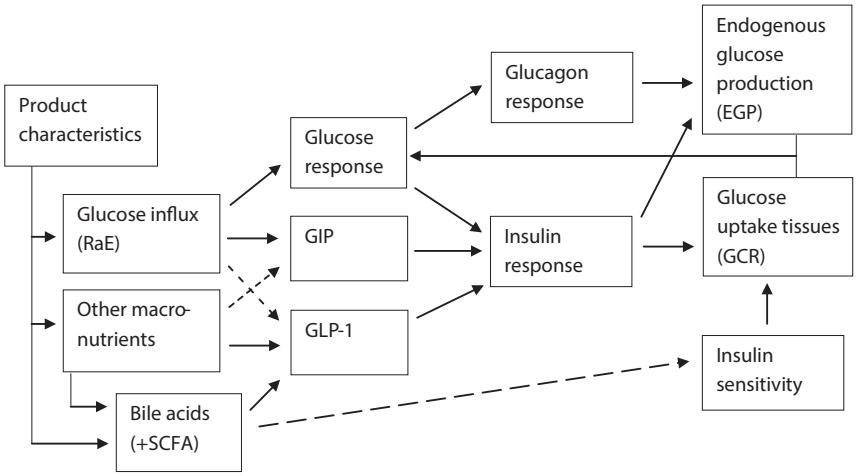


Figure 1. *Simplified scheme of metabolic interactions.*

7.3 RELEVANCE AND IMPLICATIONS OF OUR FINDINGS

The modification of our wheat products resulted in several changes in postprandial responses and metabolic interactions. The relevance and implications of the results will be discussed in this section. This will include the relevance of the observed postprandial responses for the prevention of diabetes, and the implications and opportunities related to food processing to design healthier starchy foods.

High glucose vs low insulin response

While studying the postprandial responses of several different wheat products, a remarkable finding was the discrepancy between the glucose and insulin response after consumption of products like PA and FB, and the possibility to reveal the underlying processes in this phenomenon by linking glucose kinetics and postprandial hormone responses. Although already observed after consumption of several other grain products, such as rye bread, an explanation for this lower insulin response was not yet found (11, 76). Based on our results we can assume that a slow RaE (due to e.g. slow digestion of starch, increased viscosity, a compact food structure) coincides with a modest release of GIP and insulin, keeping the GCR rather low, and allowing the glucose response to be relatively 'high'. Thus, in healthy individuals the GCR is matched to and thereby counterbalancing the RaE in such a way that postprandial glucose levels are regulated to an acceptable limit. Whether this is indeed the underlying mechanism explaining the 'rye factor' and other similar observations would need confirmation using dual isotope labeling.

Postprandial response explained by different glucose influx rates

In accordance with our findings are the results from intra-duodenal perfusion studies that compared glucose, insulin, and incretin responses during glucose infusion ranging from 1 to 4 kcal/min (21, 22). An increase in glucose load from 1 to 2 kcal/min considerably increased the glycemic response, but when rates of 3 kcal/min (22) or 4 kcal/min (21) were administered there was hardly any further increase in glycemic response. The latter was explained by the substantially greater incretin and insulin responses to the higher glucose infusion rates (21, 22). This is in line with our observations described in Chapter 3 and 4 after control bread and pasta consumption (comparable to 3–4 kcal/min vs 2 kcal/min, respectively), which also resulted in a similar glycemic response. With respect to pasta consumption, lower concentrations of GIP and insulin were necessary to regulate the glycemic response than after bread consumption. Thus, it seems that due to strict regulation of glucose concentrations in healthy individuals, the counterbalancing effect of GCR makes creating a low glycemic product challenging. To obtain a *low glycemic response* we hypothesized (Chapter 3, Figure 6; and as shown in **Figure 2**) that the RaE needs to be slower compared to the RaE after consumption of our pasta product, comparable to that of the 1-kcal/min infusion rate, eliciting a very low GIP and insulin response (21, 22). Because the GCR cannot decrease much further due to e.g. insulin-independent glucose uptake via GLUT1 (basal GCR), it may result in a low glycemic response. Other processes that could contribute to a lower glycemic response could be suppression of EGP (77) or increasing GCR (38), e.g. via insulin stimulation due to other ingredients such as protein, or via increased insulin sensitivity.

Postprandial response to different glucose influx rates in T2DM

The postprandial response to certain food products is likely different in persons with pre-diabetes or T2DM compared to healthy individuals, because this regulation is not functioning properly. As our research was performed in healthy volunteers, the results cannot be directly translated to (pre-) diabetic patients. However, the above described experiment with intra-duodenal glucose loads of 1, 2 and 4 kcal of glucose/min (21) was also performed in insulin resistant T2DM patients (78). These findings can give an indication of what the effect of a product with a slow RaE would be in this population. In short, the postprandial incretin responses were comparable between the healthy and T2DM subjects, but the insulin response was more pronounced in T2DM subjects, mainly in response to the high glucose load. However, while in healthy subjects the glucose response was similar (max 9 mmol/L) during the 2 and 4 kcal/min infusion of glucose due to regulation by increased incretin and insulin responses, the glucose response in T2DM subjects clearly increased with increasing influx rates. With 2 kcal/min the mean postprandial glucose response in T2DM reached around 13 mmol/L, with a moderate insulin response of 50 mU/L, but when infusing 4 kcal glucose per minute, mean glucose concentrations reached 15 mmol/L, with insulin concentrations increasing to around 200 mU/L (78). If the 2 kcal/min infusion of glucose would represent a product with a slower '*in vivo* starch digestibility' (RaE), such as PA or FB, consumption of these products by subjects with

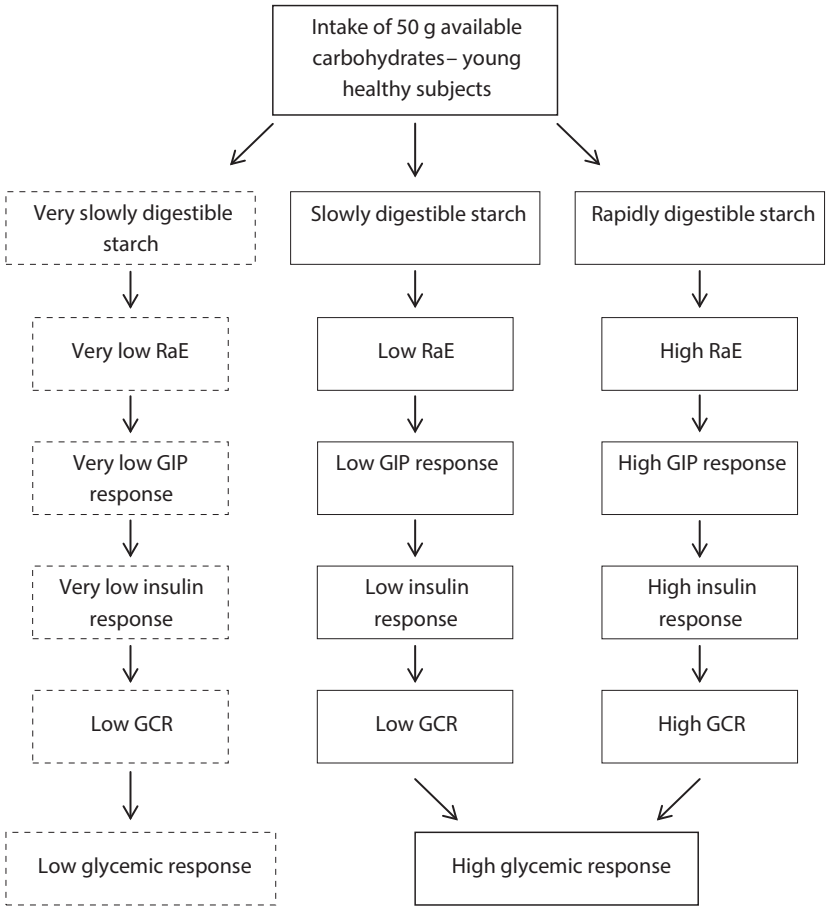


Figure 2. *Overview of the results from Chapter 3 (solid-line text boxes) comparing a rapidly and slowly digestible starchy food product (50 g available carbohydrates), which resulted in a relatively high glycemic response. On the left (dotted-line text boxes) the hypothesized situation, presumably resulting in a low glycemic response, is shown.*

IR and T2DM would likely result in a lower glucose response and a much lower insulin and incretin response compared to a product with rapid intestinal glucose influx. Therefore, we hypothesize that these products could also be useful in the management of T2DM.

Relevance for the prevention of T2DM

Lowering postprandial glucose is the main goal of pharmaceutical and nutritional treatments of T2DM, as repeated or chronic high glucose concentrations cause damage to nerves and blood vessels resulting in severe complications. In addition, lower glucose excursions may also contribute to a decreased risk of the development of metabolic diseases like T2DM. Despite general assumptions that the glycemic response reflects '*in vivo* starch digestibility', we observed that the consumption of starchy foods that elicit a slow RaE does not always result in a low glycemic response in healthy

individuals. We found similar glucose responses after consumption of our products, although this was resulting from different rates of influx of starch-derived glucose. These differences in glucose influx did however result in differences in the response of e.g. some regulating hormones. An interesting aspect is whether the observed response after consumption of products with a slow RaE, but without the desired low glucose response, might still have beneficial health effects and may contribute to the prevention of T2DM.

GIP and insulin

When glucose is absorbed by the intestinal cells at a slower rate following a meal, less GIP and insulin is secreted. GIP, mainly known for the incretin effect, has been linked to bone metabolism, suggesting positive effects of this gut hormone (79). However, there is evidence that *low* GIP concentrations could be more beneficial. Based on animal studies it is suggested that GIP might be involved in the development of obesity, due to its anabolic effects in adipose tissue, such as stimulation of fatty acid synthesis and enhancement of insulin-stimulated incorporation of fatty acids into triglycerides (80-82). In studies with mice genetically predisposed to develop obesity-related diabetes, sustained GIP receptor antagonism from early age (representing pre-diabetes) prevented the development of many of the metabolic abnormalities (83). However, despite the presence of the GIP receptor in human adipose tissue (84), the importance of these findings for the human situation is not yet elucidated as results from human studies are inconsistent (79).

In persons with pre-diabetes, GIP is secreted in normal amounts in response to an OGTT or meal (85, 86). Also in patients with T2DM, GIP secretion is generally not different compared to normal subjects (87). However, an impaired incretin effect of GIP is observed in persons with T2DM (88, 89), but not (yet) in persons with IGT (86, 90), which may be due to a gradual decrease in expression or sensitivity of the GIP receptor (91-93). This decreased effectiveness also explains why GIP receives little attention in the treatment of T2DM, as possible beneficial stimulating effects of GIP on insulin secretion to compensate for insulin resistance will not likely occur. In contrast to GLP-1, GIP may increase glucagon secretion (94), or antagonize the suppression of glucagon by GLP-1 (95), which may have an adverse effect on EGP. Thus, low postprandial GIP concentrations would be most desirable, also in the light of the possible role of GIP in fat accumulation.

Insulin is involved in many physiological processes, and crucial for the regulation of glycemic control and anabolic processes, but regular high insulin responses (hyperinsulinemia) may have adverse effects. A prospective cohort study (96) found that a higher dietary insulin index and load during puberty were associated with a higher percentage of body fat in young adulthood. In a subsequent 3 month lifestyle and metformin intervention trial in obese IR adolescents, diets with a lower insulin demand were associated with a lower energy intake which may assist with weight loss (97). Foods and/or diets resulting in a reduced postprandial insulin response are considered to induce higher satiety and a lower voluntary food intake at a subsequent meal, compared to foods with a high insulin demand (98). Rapid glucose influx will result in overstimulation of the counter-

regulatory response from the incretins and insulin, often resulting in postprandial hypoglycemia (99). In turn, this may result in craving for high GI foods, large fluctuations in glucose concentrations and overeating. A reduced postprandial insulin response to a moderate glucose influx might also be considered beneficial as it would be less demanding for the pancreatic β-cells, which may slow the process of deterioration as seen in the progression of T2DM (100, 101).

Possible role of meal-induced inflammation

The body's chronic state of low-grade inflammation is a common characteristic in obesity and related metabolic disorders like T2DM and CVD, and is associated with IR and an increased risk of the development of T2DM (102, 103). The pro-inflammatory factors in the circulation are thought to be mainly derived from macrophages in dysfunctioning adipose tissue e.g. due to chronic energy excess (104, 105).

High glucose (as well as high fat) intake (acute energy excess) can result in postprandial inflammation, which may contribute to the chronic low grade inflammation as observed in metabolic disorders, and is also increasingly implicated with both the onset of IR and atherosclerosis (106). Hyperglycemia induced by either a hyperglycemic clamp, oral glucose, or a high-GI meal has been shown to trigger an immune response via activation of nuclear transcription factor κB activity (NF-κB) in isolated peripheral blood mononuclear cells from healthy volunteers (107). Plasma concentrations of IL-6 were higher after a high-GI meal, especially in obese subjects (108), whereas higher IL-6, IL-18 and TNF-α concentrations were observed after plasma glucose concentrations were acutely raised in a glucose clamp study in both healthy volunteers and persons with IGT (109). Additionally, it was shown that hyperglycemic spikes affect cytokine concentrations more than continuous hyperglycemia. The effects were more pronounced in persons with IGT, who had higher fasting values of IL-6 and TNF-α, a higher cytokine peak after the first glucose spike and showed a longer increase in cytokine levels (4h vs 2h) during the clamp (109). In this study, concomitant intake of the antioxidant glutathione completely prevented the rise in plasma cytokines, suggesting a pronounced role for oxidative stress (109). Other studies also suggest postprandial inflammation to be mediated by oxidative stress (110, 111). Indeed, hyperglycemia can lead to the formation of reactive oxygen species (ROS) such as superoxide (112), also involved in diabetic complications such as vascular damage. As summarized by Muñoz et al., excess glucose and free fatty acids reaching the mitochondria can lead to an increased production of acetylCoA by overwhelming the TCA cycle, which overloads the electron transport chain, resulting in increased ROS within the cell, activating redox-sensitive transcription factors like NF-κB, inducing an inflammatory response (113). Among other pathways, elevated pro-inflammatory factors can induce insulin resistance by interfering with normal insulin receptor signaling, preventing the translocation of GLUT4 to the membrane and thereby the insulin-mediated uptake of glucose (106, 114, 115).

Thus, in healthy persons and persons at risk, over the long term, these meal-induced repeated inflammatory responses related to hyperglycemia may increase IR and thereby glucose

concentrations and insulin responses, resulting in a vicious cycle towards the development T2DM. It is however difficult to determine, especially from food studies, whether the glycemic response itself, or the intestinal glucose influx rate and/or other associated responses would cause these inflammatory effects. There are for instance indications that the insulin response, independent form the glycemic response, may also affect the inflammatory status. This was studied in a 12-wk intervention in individuals with the metabolic syndrome, who were placed on a rye bread and pasta diet, characterized by a low postprandial insulin response, and an oat and wheat bread and potato diet, characterized by a high postprandial insulin response (116, 117). Despite the difference in insulin responses, these products are known from previous studies to result in similar glucose responses (10, 11, 29). Whereas the 'high-insulin diet' upregulated genes related to inflammation and oxidative stress in subcutaneous adipose tissue (116), and tended to increase the inflammation markers IL-6 and IL-1β in serum, after consumption of the 'low-insulin diet' a small reduction of these inflammatory cytokines was found (117).

It is however debatable whether these findings are explained by the difference in insulin response. The products described likely differ in their '*in vivo* starch digestibility', as we observed in our studies. The low postprandial insulin response in their rye bread and pasta diet is most likely the result of a slow influx rate of glucose and a low incretin response, and resulting in a slow GCR after consumption of these products. The oat and wheat bread and potato diet can be expected to evoke a rapid influx rate of glucose and GCR. A more rapid glucose delivery, indicated by the RaE and GCR (or high insulin response), and a subsequent increased oxidation rate of glucose, could be expected to influence the amount of oxidative stress and inflammation that is evoked (112, 113, 118). In the body's attempt to lower the postprandial concentration of glucose in the blood, glucose is 'forced' into the cells, regulated by the amount of insulin. Consequently, a rapid glucose influx and high insulin response would result in more (or excess) glucose in the cell. However, high concentrations of glucose are not only harmful for blood vessels, but also for cells and tissues. If this nutrient delivery is more than the cell can process (nutrient overload), this may result in oxidative stress and inflammation (113). In this context, inflammation may be the stress signal evoked by ROS production and the associated IR could be seen as a protective mechanism for the cells to avoid further overload with glucose (119). However, it must be noted that such effects of a difference in glucose availability on inflammatory markers will be minimal, and may be difficult to observe after a meal (120), which does not rule out a cumulative effect over time that may lead to a progressive deterioration of glucose metabolism.

Hence, the intestinal influx rate of glucose may be a crucial factor, also because of the effects of the related postprandial GIP and insulin concentrations. Lowering postprandial glucose per se may (first) not be necessary, because a decreased glucose influx rate may limit mitochondrial nutrient overload, help reduce inflammation and eventually prevent IR. It is hypothesized that together, these factors may contribute to decreasing the risk of developing T2DM.

In this context, in the treatment of T2DM, it may not be wise to use medication that overrides

this protective mechanism of insulin resistance, such as synthetic insulin and insulin sensitizers, especially in obese patients with a positive energy balance (121). Lifestyle intervention (exercise and diet) is much more effective and desirable, as it addresses the root of the problem. Exercise results in a higher energy need for the muscle cells, increasing insulin sensitivity, and with respect to diet, less energy intake or *regulated release decreases the influx of glucose* and lipids into the cell, to prevent overload and oxidative stress, which also decreases insulin resistance. The same applies for persons with pre-diabetes to prevent the progression to diabetes and possibly reverse their impaired glucose tolerance to normal glucose tolerance. Products with a decreased influx of glucose (slow RaE) might be helpful for this population.

GLP-1 in the prevention of T2DM

Certain product characteristics also influenced the postprandial response mainly independent from glucose kinetics, as we found for GLP-1 after consumption of CB and KB (Chapter 5). Although with respect to the incretin GIP a low postprandial response seems most desirable, this may be somewhat different for GLP-1. The effects on health are merely beneficial, as GLP-1 is also related to e.g. decreased gastric emptying, increased satiety, and decreased food intake, and possibly β-cell preservation, as described in Chapter 1. Therefore, GLP-1 might be a valuable hormone in the fight against obesity and related diseases. Also in the light of treatment of T2DM high GLP-1 concentrations are more desirable. However, impaired postprandial GLP-1 responses in pre-diabetes and T2DM (86, 122) have been reported. In contrast, an extensive meta-analysis of the available data found no overall decreased GLP-1 response in T2DM, but observed low GLP-1 response to be mainly present in patients with less controlled T2DM (high HbA1c), and with a longer history of the disease (87). Importantly, the GLP-1 receptor does function in T2DM (88), and a high GLP-1 response would consequently still evoke an increased insulin response and suppression of glucagon, suppression of EGP, combined with increased satiety. Therefore, approaches to increase GLP-1 action for their potential therapeutic use in T2DM are being intensively studied (123). Because of the beneficial effects in T2DM patients, treatment with GLP-1 analogs was also proposed for people with pre-diabetes to prevent progression to T2DM (124, 125). Promising results were found in obese, partly pre-diabetic (~30%) subjects receiving the GLP-1 receptor agonist liraglutide for 20 weeks combined with a diet, including improved obesity-related risk factors such as weight loss and decreased blood pressure, and a reduced prevalence of pre-diabetes (126).

Increasing endogenous GLP-1 concentrations by nutrition would be interesting in relation to the prevention of T2DM. Although GLP-1 is influenced by glucose in the intestine, its release is only upregulated with a relatively high (initial) glucose influx, as demonstrated with intra-duodenal perfusion studies in both healthy subjects and T2DM patients (21, 24, 78, 127). A high glucose load is however not a desired stimulus, so it is interesting that GLP-1 secretion can be influenced, and thereby targeted, via other routes. This makes GLP-1 a relevant, but also complex and challenging target for nutritional intervention. We observed an unexpected decrease in postprandial GLP-1

concentrations after our KB bread (Chapter 5), so careful studies would be necessary addressing the exact mechanisms involved.

Plasma bile acids were found to play a role in GLP-1 stimulation as well, as discussed in section 7.2. Therapies that increase BAs (e.g. BA sequestrants or BA mimetics) in the distal bowel as a means of increasing endogenous GLP-1 concentrations have been suggested as novel treatments of T2DM and obesity (75). In our studies, the conjugated BA showed a pronounced response after consumption of the breads. Increasing or extending the postprandial BA response after ingestion of starchy food products would be an interesting approach, as besides stimulating GLP-1 excretion, BAs have a possible role in affecting insulin sensitivity and increased energy expenditure (40, 44, 45). Although the effects of BA are numerous, their role in glucose metabolism should not be underestimated. More research in this field may generate useful insights in the prevention and treatment of metabolic diseases.

Implications and opportunities related to food processing

General recommendations like reducing calories or eating more healthy and whole foods are important in the prevention of diabetes. Additionally, there are opportunities for the bread industry related to product modification. As discussed above, there are several product characteristics that potentially change the postprandial response to bread and may contribute to the prevention of T2DM.

Lowering the glycemic response is often the main goal of improving starchy foods. However, this is challenging in healthy subjects due to regulation of the glucose concentrations and may not necessarily be strived for. Positive effects of products with a slow release and/or influx of starch-derived glucose were observed as well. Products with a slow RaE could contribute to a moderate nutrient load, which may be beneficial by inducing less oxidative stress and inflammation, and thereby decreasing IR and the risk of developing diabetes. In addition, they result in lower GIP and insulin responses, less glycemic fluctuations, and likely less overeating.

Therefore it is important to design and create these starchy food products and increase the availability for consumers. There would be several ways to address this issue, for instance by changing the structure of bread. We found that a more compact bread generally results in this slower release of glucose. In our study we prepared flatbread, which was made without yeast and baked shortly with high temperature (Chapter 4). Comparable products would be a wrap, tortilla, chapatti, roti or pita bread, some of which are classified as low GI products (128), and are often well received by consumers.

A more compact structure of classical breads and baguettes could for instance be achieved by using a more traditional way of bread making, using different ways of leavening and/or kneading (12, 13). Also reducing the amount of yeast and/or applying sourdough fermentation would be a suitable option, to create a more rigid bread structure. Sourdough fermentation, often applied in rye breads, was related to reduced gastric emptying and thereby may slow starch digestibility and

decrease starch-derived glucose uptake. Related to this, sourdough may further increase solubility of the soluble fiber AX, likely influencing viscosity (37). We found AX availability in pasta and bread to be changed by processing, possibly affecting the rate of *in vivo* starch digestibility. In addition, isolated AX could be a relevant ingredient when aiming at slower glucose influx and low postprandial GIP and insulin responses, as previously observed in a study with pigs (36). Importantly, with these modifications, the palatability of the bread product must be taken into consideration, because most people prefer more soft structured ('fluffy') and light bread.

For research purposes, it is important to be able to identify these beneficial starchy food products. This would be mainly relevant in intervention studies using the glycemic response to characterize test products, e.g. investigating the physiological effects of low- and high-GI food products or diets. The products under study (e.g. with the same GI) may have a very diverse underlying glucose kinetics and postprandial hormone response, which often remains unnoticed because only the glycemic response or the food composition is known, and consequently can lead to great variances in outcome. This may also explain some of the inconsistencies observed in the literature as previously noted (129). To generate reliable, comparable and consistent results, care should be taken when characterizing test meals only based on their glycemic response, because this is the result of several underlying processes. The assessment of the metabolic quality of the starchy food products should therefore be based on additional markers. To be able to reflect the differences in RaE (which is often not determined), insulin or GIP (reflecting RaE) are advised to be taken into account as well, especially in healthy subjects.

Other routes to slow starch digestion are promising and ask for further research. The addition of natural ingredients that inhibit α-glucosidase and thereby starch digestibility (e.g. anthocyanins, Chapter 2) is still an interesting approach. However, this would need additional research related to e.g. dose and stability. The use of coarser meal or the addition of intact or broken kernels in bread making is also a possible way to decrease starch digestibility and the glycemic and/or insulinemic response (5-7), although also this approach is not always successful ((130) and Chapter 5).

It can be expected that modifications aimed at slowing the digestibility of starch or decreasing the uptake of starch-derived glucose will result in increased delivery of unabsorbed carbohydrates to the colon, resulting in fermentation by intestinal microbiota and the production of beneficial SCFA. Besides stimulating GLP-1 secretion, SCFA are known for several other beneficial health effects, related to colonic health and anti-inflammation (131, 132). An interesting finding related to weight control was that SCFA infusion into the colon, in concentrations and ratios reached after fiber intake, increased fat oxidation and energy expenditure in overweight and obese subjects (133).

The observations in Chapter 5 showed that the GLP-1 response can be influenced independently from intestinal glucose influx, due to modification of the bread product. However, it also shows that more research is necessary to elucidate the underlying mechanisms, as the addition of broken wheat kernels resulted in an unexpected decrease in GLP-1. Because of the possible effects of GLP-1 in the prevention and management of T2DM, the nutritional stimulation and extension of the GLP-1

response would be a challenging goal.

Related to this, consumption of the breads resulted in a pronounced response of BAs (Chapter 4 + 5), although it is unclear how the 'difference' in plasma BA peaks was evoked. Besides the ability to stimulate GLP-1 excretion, BAs may have a role in improving insulin sensitivity and increased energy expenditure (40, 44, 45). Therefore, augmenting or extending the postprandial BA response to starchy food products would also be an interesting approach in the prevention of T2DM. Which product characteristics could be modified to achieve this, would be an intriguing topic for future research.

Concluding remarks

Taken together, these findings reveal the complex metabolic processes determining postprandial glycemia and insulinemia after the consumption of different starchy food products. It was observed that the glycemic response does not generally reflect '*in vivo* starch digestibility' after consumption of fiber-rich wheat products in healthy men. Slower intestinal uptake of glucose from a starchy food product resulted in a lower response of gastrointestinal hormones, such as GIP, and consequently a lower insulin response. Because this low insulin response would generally also result in a slower GCR, the slow RaE does not necessarily result in a lower glycemic response. This shows that the rate of '*in vivo* starch digestion', which could be influenced by factors such as the compactness and/or the viscosity of the food product, is better reflected by the GIP and insulin response than by postprandial glycemia in healthy individuals.

Even without being able to reduce postprandial glycemia, these products may have beneficial long-term effects due to the moderate postprandial response of e.g. GIP and insulin. Slow influx of glucose may also result in less oxidative stress and inflammation, thereby reducing IR and the risk of developing T2DM. The GLP-1 response was mainly influenced independent of the glucose fluxes possibly due to the fiber/bran characteristics of the starchy food product, and may involve the BA response. It is important to realize that besides the postprandial glucose response, these factors also have an effect on health and should be taken into account when 'designing' healthy food products. Which factor is the most promising target to modulate via nutrition in the prevention of metabolic diseases like T2DM is intriguing, and deserves further study.

Based on the findings in this thesis, we can say that a more compact bread structure results in a moderate postprandial response, which may benefit health. It emphasizes the importance of the development of starchy food products with slower release and/or uptake of starch derived-glucose. This offers opportunities for bakeries to optimize their methods of baking bread to improve health, and e.g. may stimulate the more traditional ways of bread making.

To conclude, the quality of starchy food products and their impact on health should not solely be based on the glycemic response, as many other postprandial variables change simultaneously, and underlying responses may differ. Food structure and modification of bread (ingredients) can affect postprandial metabolism and health related outcomes, and is thereby an important factor to take into consideration in the prevention of the development of metabolic diseases such as T2DM.

REFERENCES

1. McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. J Agric Food Chem 2005;53:2760-6.
2. Akkarachiyasit S, Charoenlertkul P, Yibchok-Anun S, Adisakwattana S. Inhibitory activities of cyanidin and its glycosides and synergistic effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase. Int J Mol Sci 2010;11:3387-96.
3. Clegg ME, Pratt M, Meade CM, Henry JK. The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response. Br J Nutr 2011;106:335-8.
4. Fardet A, Leenhardt F, Lioger D, Scalbert A, Remesy C. Parameters controlling the glycaemic response to breads. Nutr Res Rev 2006;19:18-25.
5. Liljeberg H, Granfeldt Y, Bjorck I. Metabolic responses to starch in bread containing intact kernels versus milled flour. Eur J Clin Nutr 1992;46:561-75.
6. Holm J, Bjorck I. Bioavailability of starch in various wheat-based bread products: Evaluation of metabolic responses in healthy subjects and rate and extent of *in vitro* starch digestion. Am J Clin Nutr 1992;55:420-9.
7. Jenkins DJA, Wesson V, Wolever TMS, Jenkins AL, Kalmusky J, Guidici S, Csima A, Josse RG, Wong GS. Wholemeal versus wholegrain breads: Proportion of whole or cracked grain and the glycaemic response. Br Med J 1988;297:958-60.
8. Burton P, Lightowler HJ. Influence of bread volume on glycaemic response and satiety. Br J Nutr 2006;96:877-82.
9. Granfeldt Y, Björck I. Glycemic response to starch in pasta: a study of mechanisms of limited enzyme availability. J Cereal Sci 1991;14:47-61.
10. Juntunen KS, Niskanen LK, Liukkonen KH, Poutanen KS, Holst JJ, Mykkanen HM. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. Am J Clin Nutr 2002;75:254-62.
11. Juntunen KS, Laaksonen DE, Autio K, Niskanen LK, Holst JJ, Savolainen KE, Liukkonen KH, Poutanen KS, Mykkänen HM. Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread. Am J Clin Nutr 2003;78:957-64.
12. Bakhoj S, Flint A, Holst JJ, Tetens I. Lower glucose-dependent insulinotropic polypeptide (GIP) response but similar glucagon-like peptide 1 (GLP-1), glycaemic, and insulinaemic response to ancient wheat compared to modern wheat depends on processing. Eur J Clin Nutr 2003;57:1254-61.
13. Rizkalla SW, Laromiguiere M, Champ M, Bruzzo F, Boillot J, Slama G. Effect of baking process on postprandial metabolic consequences: Randomized trials in normal and type 2 diabetic subjects. Eur J Clin Nutr 2007;61:175-83.
14. Saulnier L, Sado P, Branlard G, Charmet G, Guillon F. Wheat arabinoxylans: exploiting variation in amount and composition to develop enhanced varieties. J Cereal Sci 2007;46:261-81.
15. EFSA Panel on Dietetic Products,Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to arabinoxylan produced from wheat endosperm and reduction of post-prandial glycaemic responses (ID 830) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9:2055.
16. Lu ZX, Walker KZ, Muir JG, Mascara T, O'Dea K. Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. Am J Clin Nutr 2000;71:1123-8.
17. Dikeman CL, Fahey Jr GC. Viscosity as related to dietary fiber: a review. Crit Rev Food Sci Nutr 2006;46:649-63.
18. Jurgoński A, Krotkiewski M, Juśkiewicz J, Billing-Marczak K. Suppression of postprandial glycaemia by L-arabinose in rats is more associated with starch than sucrose ingestion–short report. Pol J Food Nutr Sci 2015;65:57-60.
19. Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Englyst KN, Holst JJ, Stellaard F, Vonk RJ. The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic

polypeptide concentrations in healthy men. J Nutr 2006;136:1511-6.

20. Schirra J, Katschinski M, Weidmann C, Schafer T, Wank U, Arnold R, Goke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 1996;97:92-103.
21. Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Smout AJPM, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. Am J Physiol Endocrinol Metab 2007;293:E743-53.
22. Trahair LG, Horowitz M, Rayner CK, Gentilcore D, Lange K, Wishart JM, Jones KL. Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. J Clin Endocrinol Metab 2012;97:844-51.
23. Moriya R, Shirakura T, Ito J, Mashiko S, Seo T. Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. Am J Physiol Endocrinol Metab 2009;297:E1358-65.
24. O'Donovan DG, Doran S, Feinle-Bisset C, Jones KL, Meyer JH, Wishart JM, Morris HA, Horowitz M. Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. J Clin Endocrinol Metab 2004;89:3431-5.
25. Holt SHA, Brand Miller JC, Petocz P. An insulin index of foods: The insulin demand generated by 1000-kJ portions of common foods. Am J Clin Nutr 1997;66:1264-76.
26. Björck I, Liljeberg H, Ostman E. Low glycaemic-index foods. Br J Nutr 2000;83 Suppl 1:S149-55.
27. Lin MH, Wu MC, Lu S, Lin J. Glycemic index, glycemic load and insulinemic index of Chinese starchy foods. World J Gastroenterol 2010;16:4973-9.
28. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 1986;63:492-8.
29. Leinonen K, Liukkonen K, Poutanen K, Uusitupa M, Mykkänen H. Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. Eur J Clin Nutr 1999;53:262-7.
30. Östman E, Granfeldt Y, Persson L, Björck I. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. Eur J Clin Nutr 2005;59:983-8.
31. Liljeberg HG, Bjorck IM. Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: studies in humans and rats using test products with added organic acids or an organic salt. Am J Clin Nutr 1996;64:886-93.
32. Hoebler, M.-F. Devaux, A. Karinthi, C. Belleville, J.-L. Barry, C. Particle size of solid food after human mastication and *in vitro* simulation of oral breakdown. Int J Food Sci Nutr 2000;51:353-66.
33. Nordlund E, Katina K, Mykkänen H, Poutanen K. Distinct characteristics of rye and wheat breads impact on their *in vitro* gastric disintegration and *in vivo* glucose and insulin responses. Foods 2016;5:24.
34. Bornhorst GM, Singh RP. Bolus formation and disintegration during digestion of food carbohydrates. Comprehensive Reviews in Food Science and Food Safety 2012;11:101-18.
35. Knudsen KEB, Lærke HN. Rye arabinoxylans: molecular structure, physicochemical properties and physiological effects in the gastrointestinal tract. Cereal Chem 2010;87:353-62.
36. Christensen KL, Hedemann MS, Lærke HN, Jørgensen H, Mutt SJ, Herzig K, Bach Knudsen KE. Concentrated arabinoxylan but not concentrated β-glucan in wheat bread has similar effects on postprandial insulin as whole-grain rye in porto-arterial catheterized pigs. J Agric Food Chem 2013;61:7760-8.
37. Lappi J, Selinheimo E, Schwab U, Katina K, Lehtinen P, Mykkänen H, Kolehmainen M, Poutanen K. Sourdough fermentation of wholemeal wheat bread increases solubility of arabinoxylan and protein and decreases postprandial glucose and insulin responses. J Cereal Sci 2010;51:152-8.
38. Schenk S, Davidson CJ, Zderic TW, Byerley LO, Coyle EF. Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am J Clin Nutr 2003;78:742-8.
39. Ørskov L, Holst J, Møller J, Ørskov C, Møller N, Alberti K, Schmitz O. GLP-1 does not acutely affect insulin

sensitivity in healthy man. Diabetologia 1996;39:1227-32.

40. Cariou B, van Harmelen K, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, Caron S, Torpier G, Fruchart JC, Gonzalez FJ, et al. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. J Biol Chem 2006;281:11039-49.
41. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM, Edwards PA. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci U S A 2006;103:1006-11.
42. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 2006;116:1102-9.
43. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 2006;313:1137-40.
44. Kars M, Yang L, Gregor MF, Mohammed BS, Pietka TA, Finck BN, Patterson BW, Horton JD, Mittendorfer B, Hotamisligil GS, et al. Tauroursodeoxycholic Acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. Diabetes 2010;59:1899-905.
45. van Nierop FS, Scheltema MJ, Eggink HM, Pols TW, Sonne DP, Knop FK, Soeters MR. Clinical relevance of the bile acid receptor TGR5 in metabolism. The lancet Diabetes & endocrinology 2017;5:224-33.
46. Cherrington A, Edgerton D, Sindelar D. The direct and indirect effects of insulin on hepatic glucose production *in vivo*. Diabetologia 1998;41:987-96.
47. Priebe MG, Wachters-Hagedoorn RE, Heimweg JA, Small A, Preston T, Elzinga H, Stellaard F, Vonk RJ. An explorative study of *in vivo* digestive starch characteristics and postprandial glucose kinetics of wholemeal wheat bread. Eur J Nutr 2008;47:417-23.
48. Larsson H, Holst JJ, Ahr+¬n B. Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. Acta Physiol Scand 1997;160:413-22.
49. Prigeon RL, Quddusi S, Paty B, D'Alessio DA. Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. Am J Physiol Endocrinol Metab 2003;285:E701-7.
50. Bavenholm PN, Pigon J, Ostenson CG, Efendic S. Insulin sensitivity of suppression of endogenous glucose production is the single most important determinant of glucose tolerance. Diabetes 2001;50:1449-54.
51. Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM. Human duodenal enteroendocrine cells: Source of both incretin peptides, GLP-1 and GIP. American Journal of Physiology - Endocrinology and Metabolism 2006;290:E550-9.
52. Gorboulev V, Schurmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, et al. Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes 2012;61:187-96.
53. Powell DR, Smith M, Greer J, Harris A, Zhao S, DaCosta C, Mseeh F, Shadoan MK, Sands A, Zambrowicz B, et al. LX4211 increases serum glucagon-like peptide 1 and peptide YY levels by reducing sodium/glucose cotransporter 1 (SGLT1)-mediated absorption of intestinal glucose. J Pharmacol Exp Ther 2013;345:250-9.
54. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 2012;61:364-71.
55. Qualmann C, Nauck MA, Holst JJ, Ørskov C, Creutzfeldt W. Glucagon-like peptide 1 (7-36 amide) secretion in response to luminal sucrose from the upper and lower gut. A study using α-glucosidase inhibition (acarbose). Scand J Gastroenterol 1995;30:892-6.
56. Seifarth C, Bergmann J, Holst JJ, Ritzel R, Schmiegel W, Nauck MA. Prolonged and enhanced secretion of glucagon-like peptide 1 (7-36 amide) after oral sucrose due to alpha-glucosidase inhibition (acarbose) in Type 2 diabetic patients. Diabet Med 1998;15:485-91.
57. Verbeke K, Ferchaud-Roucher V, Preston T, Small AC, Henckaerts L, Krempf M, Wang H, Vonk RJ, Priebe MG. Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in

healthy human volunteers. Eur J Clin Nutr 2010;64:678-84.

58. Jenkins DJA, Kendall CWC, Vuksan V, Augustin LSA, Li Y-, Lee B, Mehling CC, Parker T, Faulkner D, Seyler H, et al. The effect of wheat bran particle size on laxation and colonic fermentation. J Am Coll Nutr 1999;18:339-45.
59. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun 2005;329:386-90.
60. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009;10:167-77.
61. Rafferty EP, Wylie AR, Hand KH, Elliott CE, Grieve DJ, Green BD. Investigating the effects of physiological bile acids on GLP-1 secretion and glucose tolerance in normal and GLP-1R -/- mice. Biol Chem 2011;392:539-46.
62. Parker HE, Wallis K, le Roux CW, Wong KY, Reimann F, Gribble FM. Molecular mechanisms underlying bile acid-stimulated glucagon-like peptide-1 secretion. Br J Pharmacol 2012;165:414-23.
63. Wu T, Bound MJ, Standfield SD, Jones KL, Horowitz M, Rayner CK. Effects of Taurocholic Acid on Glycemic, Glucagon-like Peptide-1, and Insulin Responses to Small Intestinal Glucose Infusion in Healthy Humans. J Clin Endocrinol Metab 2013;98:E718-22.
64. Matysik S, Martin J, Bala M, Scherer M, Schaffler A, Schmitz G. Bile acid signaling after an oral glucose tolerance test. Chem Phys Lipids 2011;164:525-9.
65. Ho JE, Larson MG, Vasan RS, Ghorbani A, Cheng S, Rhee EP, Florez JC, Clish CB, Gerszten RE, Wang TJ. Metabolite profiles during oral glucose challenge. Diabetes 2013;62:2689-98.
66. Roberts RE, Glicksman C, Alaghband-Zadeh J, Sherwood RA, Akuji N, le Roux CW. The relationship between postprandial bile acid concentration, GLP-1, PYY and ghrelin. Clin Endocrinol (Oxf) 2011;74:67-72.
67. Sonne DP, Hare KJ, Martens P, Rehfeld JF, Holst JJ, Vilsboll T, Knop FK. Postprandial gut hormone responses and glucose metabolism in cholecystectomized patients. Am J Physiol Gastrointest Liver Physiol 2013;304:G413-9.
68. Hopman W, Jansen J, Lamers C. Comparative study of the effects of equal amounts of fat, protein, and starch on plasma cholecystokinin in man. Scand J Gastroenterol 1985;20:843-7.
69. Bourdon I, Olson B, Backus R, Richter BD, Davis PA, Schneeman BO. Beans, as a source of dietary fiber, increase cholecystokinin and apolipoprotein b48 response to test meals in men. J Nutr 2001;131:1485-90.
70. Burton-Freeman B, Davis PA, Schneeman BO. Plasma cholecystokinin is associated with subjective measures of satiety in women. Am J Clin Nutr 2002;76:659-67.
71. Beck EJ, Tosh SM, Batterham MJ, Tapsell LC, Huang X. Oat β-glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. Molecular nutrition & food research 2009;53:1343-51.
72. Story JA, Kritchevsky D. Comparison of the binding of various bile acids and bile salts *in vitro* by several types of fiber. J Nutr 1976;106:1292-4.
73. Beysen C, Murphy EJ, Deines K, Chan M, Tsang E, Glass A, Turner SM, Protasio J, Riiff T, Hellerstein MK. Effect of bile acid sequestrants on glucose metabolism, hepatic de novo lipogenesis, and cholesterol and bile acid kinetics in type 2 diabetes: a randomised controlled study. Diabetologia 2012;55:432-42.
74. Harach T, Pols TW, Nomura M, Maida A, Watanabe M, Auwerx J, Schoonjans K. TGR5 potentiates GLP-1 secretion in response to anionic exchange resins. Sci Rep 2012;2:430.
75. Adrian TE, Gariballa S, Parekh KA, Thomas SA, Saadi H, Al Kaabi J, Nagelkerke N, Gedulin B, Young AA. Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in obese type 2 diabetic volunteers. Diabetologia 2012;55:2343-7.
76. Rosen LA, Silva LO, Andersson UK, Holm C, Ostman EM, Bjorck IM. Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile. Nutr J 2009;8:42.
77. Boers HM, van Dijk TH, Hiemstra H, Hoogenraad A, Mela DJ, Peters HP, Vonk RJ, Priebe MG. Effect of fibre

additions to flatbread flour mixes on glucose kinetics: a randomised controlled trial. Br J Nutr 2017;118:777-87.

78. Ma J, Pilichiewicz A, Feinle-Bisset C, Wishart J, Jones K, Horowitz M, Rayner C. Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes. Diabetic Med 2012;29:604-8.

79. Holst JJ, Windeløv JA, Boer GA, Pedersen J, Svendsen B, Christensen M, Torekov S, Asmar M, Hartmann B, Nissen A. Searching for the physiological role of glucose-dependent insulinotropic polypeptide. Journal of diabetes investigation 2016;7:8-12.

80. Yip RGC, Wolfe MM. GIP biology and fat metabolism. Life Sci 2000;66:91-103.

81. Song DH, Wolfe MM. Glucose-dependent insulinotropic polypeptide and its role in obesity. Curr Opin in Endocrinol Diabetes Obes 2007;14:46-51.

82. Paschetta E, Hvalryg M, Musso G. Glucose-dependent insulinotropic polypeptide: from pathophysiology to therapeutic opportunities in obesity-associated disorders. Obes Rev 2011;12:813-28.

83. Irwin N, McClean P, O'harte F, Gault V, Harriott P, Flatt P. Early administration of the glucose-dependent insulinotropic polypeptide receptor antagonist (Pro3) GIP prevents the development of diabetes and related metabolic abnormalities associated with genetically inherited obesity in ob/ob mice. Diabetologia 2007;50:1532-40.

84. Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 1993;133:2861-70.

85. Laakso M, Zilinskaite J, Hansen T, Boesgaard TW, Vänttinen M, Stančáková A, Jansson P, Pellmé F, Holst J, Kuulasmaa T. Insulin sensitivity, insulin release and glucagon-like peptide-1 levels in persons with impaired fasting glucose and/or impaired glucose tolerance in the EUGENE2 study. Diabetologia 2008;51:502-11.

86. Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ, Ferrannini E. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes 2008;57:1340-8.

87. Calanna S, Christensen M, Holst JJ, Laferrere B, Gluud L, Vilsbøll T, Knop F. Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. Diabetologia 2013;56:965-72.

88. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993;91:301-7.

89. Vilsbøll T, Krarup T, Madsbad S, Holst J. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. Diabetologia 2002;45:1111-9.

90. Muscelli E, Mari A, Natali A, Astiarraga BD, Camastra S, Frascerra S, Holst JJ, Ferrannini E. Impact of incretin hormones on β-cell function in subjects with normal or impaired glucose tolerance. American Journal of Physiology-Endocrinology and Metabolism 2006;291:E1144-50.

91. Holst JJ, Gromada J, Nauck M. The pathogenesis of NIDDM involves a defective expression of the GIP receptor. Diabetologia 1997;40:984-6.

92. Lynn FC, Pamir N, Ng EH, McIntosh CH, Kieffer TJ, Pederson RA. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. Diabetes 2001;50:1004-11.

93. Zhou J, Livak MF, Bernier M, Muller DC, Carlson OD, Elahi D, Maudsley S, Egan JM. Ubiquitination is involved in glucose-mediated downregulation of GIP receptors in islets. Am J Physiol Endocrinol Metab 2007;293:E538-47.

94. Lund A, Vilsbøll T, Bagger JI, Holst JJ, Knop FK. The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes. American Journal of Physiology-Endocrinology and Metabolism 2011;300:E1038-46.

95. Mentis N, Vardarli I, Köthe LD, Holst JJ, Deacon CF, Theodorakis M, Meier JJ, Nauck MA. GIP does not

potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. Diabetes 2011;60:1270-6.

96. Joslowski G, Goletzke J, Cheng G, Günther A, Bao J, Brand-Miller J, Buyken A. Prospective associations of dietary insulin demand, glycemic index, and glycemic load during puberty with body composition in young adulthood. Int J Obes 2012;36:1463-71.
97. Joslowski G, Halim J, Goletzke J, Gow M, Ho M, Louie JC, Buyken AE, Cowell CT, Garnett SP. Dietary glycemic load, insulin load, and weight loss in obese, insulin resistant adolescents: RESIST study. Clinical Nutrition 2015;34:89-94.
98. Rosén LA, Östman EM, Björck IM. Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; focusing on rye products. Nutr J 2011;10:7.
99. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 2002;287:2414-23.
100. Levy J, Atkinson A, Bell P, McCance D, Hadden D. Beta-cell deterioration determines the onset and rate of progression of secondary dietary failure in type 2 diabetes mellitus: the 10-year follow-up of the Belfast Diet Study. Diabetic Med 1998;15:290-6.
101. Kahn SE. The importance of β-cell failure in the development and progression of type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism 2001;86:4047-58.
102. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005;115:1111-9.
103. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860.
104. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008;29:2959-71.
105. Fain JN. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. Mediators Inflamm 2010;2010:Article ID 513948.
106. Dandona P, Ghanim H, Chaudhuri A, Dhindsa S, Kim SS. Macronutrient intake induces oxidative and inflammatory stress: potential relevance to atherosclerosis and insulin resistance. Exp Mol Med 2010;42:245-53.
107. Schiekofer S, Andrassy M, Chen J, Rudofsky G, Schneider J, Wendt T, Stefan N, Humpert P, Fritsche A, Stumvoll M, et al. Acute hyperglycemia causes intracellular formation of CML and activation of ras, p42/44 MAPK, and nuclear factor kappaB in PBMCs. Diabetes 2003;52:621-33.
108. Manning PJ, Sutherland WH, McGrath MM, de Jong SA, Walker RJ, Williams MJ. Postprandial cytokine concentrations and meal composition in obese and lean women. Obesity (Silver Spring) 2008;16:2046-52.
109. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002;106:2067-72.
110. Ceriello A, Bortolotti N, Motz E, Crescentini A, Lizzio S, Russo A, Tonutti L, Taboga C. Meal-generated oxidative stress in type 2 diabetic patients. Diabetes Care 1998;21:1529-33.
111. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006;295:1681-7.
112. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.
113. Muñoz A, Costa M. Nutritionally mediated oxidative stress and inflammation. Oxidative medicine and cellular longevity 2013;2013:Article ID 610950.
114. Qatanani M, Lazar MA. Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev 2007;21:1443-55.
115. Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science 2013;339:172-7.

116. Kallio P, Kolehmainen M, Laaksonen DE, Kekäläinen J, Salopuro T, Sivenius K, Pulkkinen L, Mykkänen HM, Niskanen L, Uusitupa M, et al. Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome: the FUNGENUT Study. Am J Clin Nutr 2007;85:1417-27.

117. Kallio P, Kolehmainen M, Laaksonen DE, Pulkkinen L, Atalay M, Mykkänen H, Uusitupa M, Poutanen K, Niskanen L. Inflammation markers are modulated by responses to diets differing in postprandial insulin responses in individuals with the metabolic syndrome. Am J Clin Nutr 2008;87:1497-503.

118. Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radical Biology and Medicine 2011;50:567-75.

119. Hoehn KL, Salmon AB, Hohnen-Behrens C, Turner N, Hoy AJ, Maghzal GJ, Stocker R, Van Remmen H, Kraegen EW, Cooney GJ, et al. Insulin resistance is a cellular antioxidant defense mechanism. Proc Natl Acad Sci U S A 2009;106:17787-92.

120. Lambert-Porcheron S, Normand S, Blond E, Sothier M, Roth H, Meynier A, Vinoy S, Laville M, Nazare J. Modulation of starch digestibility in breakfast cereals consumed by subjects with metabolic risk: impact on markers of oxidative stress and inflammation during fasting and the postprandial period. Mol Nutr Food Res 2017;61:mnfr.201700212.

121. Nolan CJ, Ruderman NB, Kahn SE, Pedersen O, Prentki M. Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. Diabetes 2015;64:673-86.

122. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes 2001;50:609-13.

123. Ahrén B, Schmitz O. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. Hormone and metabolic research 2004;36:867-76.

124. DeFronzo RA, Abdul-Ghani M. Type 2 diabetes can be prevented with early pharmacological intervention. Diabetes Care 2011;34:S202-9.

125. Holst JJ, Deacon CF. Is there a place for incretin therapies in obesity and prediabetes? Trends Endocrinol Metab 2013;24:145-52.

126. Astrup A, Rössner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim M, Madsen J, Rasmussen MF, Lean ME, NN8022-1807 Study Group. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. The Lancet 2009;374:1606-16.

127. Schirra J, Katschinski M, Weidmann C, Schäfer T, Wank U, Arnold R, Göke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 1996;97:92-103.

128. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care 2008;31:2281-3.

129. Blaak EE, Antoine JM, Benton D, Bjorck I, Bozzetto L, Brouns F, Diamant M, Dye L, Hulshof T, Holst JJ, et al. Impact of postprandial glycaemia on health and prevention of disease. Obes Rev 2012;13:923-84.

130. Behall KM, Scholfield DJ, Hallfrisch J. The effect of particle size of whole-grain flour on plasma glucose, insulin, glucagon and thyroid-stimulating hormone in humans. J Am Coll Nutr 1999;18:591-7.

131. Wong JMW, De Souza R, Kendall CWC, Emam A, Jenkins DJA. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol 2006;40:235-43.

132. Meijer K, De Vos P, Priebe MG. Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? Curr Opin Clin Nutr Metab Care 2010;13:715-21.

133. Canfora EE, van der Beek CM, Jocken JWE, Goossens GH, Holst JJ, Olde Damink SWM, Lenaerts K, Dejong CHC, Blaak EE. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. Sci Rep 2017;7:Article number: 2360.

Addendum

Summary

SUMMARY

There is an increasing prevalence of chronic metabolic disorders like obesity, insulin resistance (IR) and type 2 diabetes (T2DM). Because these disorders are highly lifestyle related, preventing the development of T2DM or slowing the progression from IR and pre-diabetes to T2DM could be targeted by nutritional strategies. Repeated high blood glucose concentrations after the consumption of carbohydrate-rich foods, a high postprandial glycemic response, is one of the factors that may increase the risk for development of IR and T2DM. Starchy food products, such as bread and pasta, which are frequently consumed world-wide, differ substantially in their physiological and metabolic response ('metabolic quality') depending on their product characteristics. Improving the postprandial response to these food products, e.g. by decreasing starch digestibility, might be beneficial for the prevention of T2DM. Therefore, we investigated the glucose kinetics and metabolic responses after consumption of various starchy food products. By varying with food ingredients and structure, we aimed to create slowly digestible starchy food products, to gain insight into the underlying postprandial processes accompanying the glycemic response and the possible relationship with the development of insulin resistance and T2DM. These insights are relevant in order to develop beneficial starchy food products that aid the prevention (and/or management) of these metabolic disorders.

This thesis describes the results of several nutritional interventions performed in healthy male volunteers consuming a variety of fiber-rich wheat products with different ingredients and/or structural features: pasta, bread, bread with polyphenol-rich bran, bread with 85% broken wheat kernels and flat bread. The different wheat products, enriched with the stable isotope ^{13}C, were consumed while receiving a primed-continuous D-[6,6-$^{2}H_{2}$]glucose infusion. This 'dual-isotope technique' enables the calculation of glucose kinetics, such as the rate of appearance of exogenous glucose (RaE), endogenous glucose production (EGP), and glucose clearance rate (GCR). Postprandial plasma concentrations of total glucose, and the pancreatic hormones insulin and glucagon, were investigated and related to the glucose kinetics data. The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), released from the intestinal K- and L-cells in response to food intake and involved in potentiating the glucose-induced insulin response, were taken into account as well. In addition, cholecystokinin and bile acids in plasma were analyzed, because of the increasingly recognized role of bile acids in glucose metabolism. This gave us insight into the postprandial metabolic changes that occur after consumption of starchy food products.

First we compared the postprandial response of a normal fiber-rich wheat bread and a fiber-rich pasta, with an expected high and low glycemic response, respectively. However, bread and pasta consumption resulted in a similar glycemic response in healthy men, despite a much slower influx rate of exogenous glucose (RaE) after pasta consumption. These findings emphasize that the glucose response does not only reflect the intestinal influx of starch-derived glucose from the food product,

but is the net result of several metabolic processes occurring simultaneously. Interestingly, despite the similar glucose responses, insulin concentrations were markedly lower after pasta consumption. We observed that the slower RaE after consumption of pasta compared to bread coincided with a lower postprandial GIP response. Consequently, insulin concentrations were lower, resulting in a slow GCR. This explained the relatively high glycemic response despite a slow RaE. In order to obtain a low glycemic response, we expect that the RaE after a certain starchy food product needs to be lower, without a further decrease in GCR.

From metabolomics analyses of plasma samples after consumption of bread and pasta it appeared that the slow appearance of starch-derived glucose (RaE) after pasta intake may partly result from increased viscosity. The concentrations of arabinose and xylose, originating from the viscous fiber arabinoxylan (AX), were higher after pasta consumption, although the fluxes of all other nutrients and metabolites after pasta were lower. This suggests that AX from pasta was better available for degradation in the intestine (possibly caused by hydrolyses by the small intestinal microbiota) due to processing, possibly resulting in an increased viscosity of the meal bolus. This could have induced slower gastric emptying, decreased digestibility of starch and/or interference with uptake of glucose and other nutrients.

Slow release or uptake of glucose from pasta and the resulting low GIP and insulin response might also be due to the compact food structure. Other breads made from rye and wheat with a more rigid structure showed comparable postprandial results; a normal glycemic response and a low insulin response. Therefore, we made compact flat breads, and compared the postprandial response to that of normal fiber-rich wheat bread ('control bread') and pasta. All products had the same composition (except yeast), but a different food structure due to the processing conditions. Consumption of compact flat bread resulted in a more moderate postprandial response compared to control bread. As hypothesized, the response was similar to pasta based on glucose, insulin, and the GIP response, although the RaE was slower only in the first hour postprandial. Interestingly, the GCR after flat bread was higher than expected based on the insulin response, suggesting increased insulin sensitivity or insulin-independent glucose disposal.

The effect of specific ingredients on starch digestibility and/or the glycemic response was also investigated. Bread with added purple bran was made in an attempt to slow starch digestion, because anthocyanins present in purple wheat have shown inhibitory activity toward pancreatic α-amylase in vitro. However, the purple wheat bran in the wheat bread had no effect on the rate of 'in vivo starch digestion', reflected by the similar RaE compared to the control bread. Against expectations, bread with the addition of 85% broken wheat kernels did not affect the glycemic response nor the glucose kinetics either, indicating similar starch digestibility compared to control bread. Interestingly, although no clear difference in GIP response was observed, the GLP-1 response was much lower after kernel bread compared to control bread. Thus, as observed in all studies, the

RaE showed a strong correlation with GIP, but in contrast, for GLP-1 this relationship was less clear. Despite similar glucose kinetics after control bread and kernel bread, the GLP-1 response was clearly influenced by the presence of broken wheat kernels. Possibly, stimulation of GLP-1 by bile acids may play a role in this regard.

Taken together, these findings reveal the complex metabolic processes determining postprandial glycemia and insulinemia after the consumption of starchy food products. It was observed that the glycemic response does not generally reflect 'in vivo starch digestibility' after consumption of fiber-rich wheat products in healthy men. Slower intestinal uptake of glucose from a starchy food product resulted in lower postprandial GIP and insulin concentrations, but not necessarily in a lower glycemic response, because of a slower GCR. This shows that the rate of 'in vivo starch digestion', which could be influenced by factors such as the compactness and/or the viscosity of the food product, is better reflected by the GIP and insulin response than by postprandial glycemia in healthy individuals. Even without being able to reduce postprandial glycemia, these products may have beneficial long-term effects due to the moderate postprandial response of e.g. GIP and insulin. Slow influx of glucose may also result in less oxidative stress and low-grade inflammation, thereby reducing IR and the risk of developing T2DM. The GLP-1 response was additionally influenced independent of the glucose fluxes, possibly due to other characteristics of the starchy food product, and may be related to bile acids. This deserves further study, as GLP-1 and bile acids are seen as promising therapeutic agents in T2DM and its prevention.

Based on the findings in this thesis, we state that a more compact bread structure results in a moderate postprandial response, which may benefit health. It emphasizes the importance of the development of starchy food products with slower release and/or uptake of starch-derived glucose. This offers opportunities for bakeries to optimize their methods of baking bread to improve health, and may for instance stimulate more traditional ways of bread making.

To conclude, the quality of starchy food products and their impact on health should not solely be based on the glycemic response, as many other postprandial variables change simultaneously, and underlying responses may differ. We therefore suggest to measure simultaneously insulin and incretin hormones. Modification of bread structure and ingredients can affect postprandial metabolism and health related outcomes. Thereby these are important factors to take into consideration in the prevention of the development of metabolic diseases such as T2DM.

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Addendum

Samenvatting

SAMENVATTING

Chronische metabole aandoeningen, zoals obesitas, insuline resistentie (IR) en type 2 diabetes (T2DM) komen steeds vaker voor. De ontwikkeling van deze aandoeningen is sterk gerelateerd aan leefstijl. Voeding kan dan ook een belangrijke rol spelen in het ontstaan en de preventie ervan. Herhaaldelijk hoge glucoseconcentraties in het bloed na de consumptie van koolhydraatrijke voeding, ofwel een hoge postprandiale glycemische respons, is een van de factoren die het risico op het ontstaan van IR en T2DM mogelijk kunnen verhogen. Ook zetmeelrijke voedingsmiddelen, zoals brood en pasta, die wereldwijd veelvuldig worden geconsumeerd, kunnen aanzienlijk verschillen in hun glycemische respons, afhankelijk van de productkenmerken. Het verbeteren van de postprandiale respons van deze producten, bijvoorbeeld door de verteerbaarheid van zetmeel te vertragen of te verminderen, zou gunstig kunnen zijn in de preventie van T2DM. Daarom onderzochten we de postprandiale glucosekinetiek en bijkomende postprandiale responsen die optraden na de consumptie van verschillende zetmeelrijke voedingsproducten, wat inzicht geeft in de 'metabole kwaliteit' van deze producten. Door te variëren met de ingrediënten en structuur van de producten hebben we getracht langzaam verteerbare zetmeelrijke producten te creëren, om daarmee inzicht te krijgen in de onderliggende postprandiale processen die gepaard gaan met de glycemische respons en de mogelijke relatie met het ontstaan van IR en T2DM. Deze inzichten zijn relevant om verbeterde zetmeelrijke voedingsproducten te ontwikkelen die kunnen helpen in de preventie (en/of het management) van deze metabole aandoeningen.

Dit proefschrift beschrijft de resultaten van verschillende voedingsinterventies in gezonde mannelijke vrijwilligers, die een verscheidenheid aan vezelrijke tarweproducten consumeerden met verschillende ingrediënten en/of structurele kenmerken: pasta, brood, brood met polyfenolrijke zemelen, brood met 85% gebroken tarwekorrels en plat brood. De verschillende tarweproducten, verrijkt met het stabiele isotoop ^{13}C, werden geconsumeerd door de deelnemers, terwijl ze tegelijkertijd een infusie van D-[6,6-$^{2}H_{2}$]glucose kregen. Deze 'dubbele isotoop techniek' maakt de berekening van de glucose kinetiek mogelijk, zoals de verschijningssnelheid van exogene glucose (RaE), endogene glucose productie (EGP), en de glucose klaringssnelheid (GCR). Postprandiale plasmaconcentraties van totaal glucose, en de alvleesklierhormonen insuline en glucagon, werden onderzocht en gerelateerd aan glucose kinetiek data. De incretinehormonen glucose-afhankelijk insulinotroop polypeptide (GIP) en glucagon-achtig peptide-1 (GLP-1), die vrijgemaakt worden vanuit de K- en L-cellen in de darm als reactie op voedselinname en betrokken zijn bij het versterken van de glucose-geïnduceerde insulinerespons, werden ook onderzocht. Daarnaast werden cholecystokinine en galzouten in plasma geanalyseerd, vanwege de steeds meer erkende rol van galzouten in het glucosemetabolisme. Dit gaf ons inzicht in de postprandiale metabole veranderingen die optreden na de consumptie van zetmeelrijke producten.

Allereerst vergeleken we de postprandiale respons na consumptie van een normaal vezelrijk tarwebrood en een vezelrijke pasta, met een verwachte hoge en lage glycemische respons. De consumptie van brood en pasta resulteerde echter in een vergelijkbare glycemische respons bij gezonde mannen, ondanks een veel langzamere influxsnelheid van exogene glucose (RaE) na pasta consumptie. Deze bevindingen benadrukken dat de glucoserespons niet alleen de influx van exogene glucose uit de darm reflecteert, maar het een netto resultaat is van verschillende metabole processen die tegelijkertijd plaatsvinden. Interessant is dat ondanks de vergelijkbare glycemische respons na brood- en pastaconsumptie, de insuline concentraties aanzienlijk lager waren na pastaconsumptie. We zagen dat de langzame(re) RaE na de consumptie van pasta vergeleken met brood samenging met een lage(re) postprandiale GIP-respons. Zodoende waren de insulineconcentraties ook lager, wat resulteerde in een langzame GCR. Dit verklaarde de relatief hoge glycemische respons ondanks een langzame RaE. Om een lage glycemische respons te krijgen, verwachten we dat de RaE na de consumptie van een zetmeelrijk voedingsproduct nog lager zou moeten zijn, zonder een verdere afname in GCR.

Om meer informatie te verzamelen over de onderliggende mechanismen werd onderzoek verricht aan de afbraakproducten van onder andere de voeding in het bloed, ook wel metabolieten genoemd (metabolomics). Uit deze metabolomics analyses van plasmamonsters na de consumptie van brood en pasta bleek dat de langzame verschijning van zetmeel-afkomstige glucose (RaE) na pastaconsumptie deels het resultaat zou kunnen zijn van een toename in viscositeit. De concentraties van arabinose en xylose, afkomstig van de viskeuze vezel arabinoxylan (AX), waren hoger na pastaconsumptie, hoewel de fluxen van alle andere nutriënten en metabolieten na pasta lager waren. Dit suggereert dat AX in pasta beter beschikbaar was voor afbraak in de darm (mogelijk veroorzaakt door hydrolyse door de microbiota in de dunne darm) als gevolg van de productbereiding, mogelijk resulterend in een verhoogde viscositeit van de maaltijdbolus. Dit kan geleid hebben tot langzamere maaglediging, een verminderde verteerbaarheid van zetmeel, en/of belemmering van de opname van glucose of andere nutriënten.

Langzame afgifte of opname van glucose uit pasta en de resulterende lage GIP- en insulinerespons kunnen ook veroorzaakt worden door de compacte structuur van de voeding. Andere type broden gemaakt van rogge en tarwe met een meer rigide structuur resulteerden in vergelijkbare postprandiale uitkomsten; een normale glycemische respons en een lage insulinerespons. Daarom hebben we compacte platte broden gemaakt, en hebben de postprandiale respons vergeleken met die van normaal vezelrijk tarwebrood ('controlebrood') en pasta. Alle producten hadden dezelfde samenstelling (met uitzondering van gist), maar een verschillende structuur vanwege de verschillen in bereiding. De consumptie van compact plat brood resulteerde in een meer gematigde postprandiale respons in vergelijking met het controlebrood. Zoals verondersteld

was de respons van het compacte platte brood vergelijkbaar met de respons na pasta gebaseerd op de glucose, insuline en GIP-respons, hoewel de RaE alleen vergelijkbaar langzamer was in het eerste uur na de maaltijd. Interessant is dat de GCR na plat brood hoger was dan verwacht op basis van de insulinerespons, hetgeen een verhoogde insuline gevoeligheid of insuline-onafhankelijke glucoseklaring suggereert.

Het effect van specifieke ingrediënten op de verteerbaarheid van zetmeel en/of de glycemische respons werd ook onderzocht. In een poging om de zetmeelvertering te vertragen werd brood met toegevoegde paarse zemelen gemaakt, omdat anthocyanen aanwezig in paarse tarwe een remmende activiteit hebben laten zien tegen pancreatische α-amylase in vitro. De paarse tarwezemelen in het brood hadden echter geen effect op de snelheid van 'in vivo zetmeelvertering', af te lezen aan de vergelijkbare RaE vergeleken met het controlebrood. Tegen de verwachting in had brood met de toevoeging van 85% gebroken tarwekorrels ook geen effect op de glycemische respons en de glucose kinetiek vergeleken met het controlebrood, wat op vergelijkbare verteerbaarheid van zetmeel duidt. Interessant is dat, hoewel er geen duidelijk verschil in GIP-respons werd waargenomen, de GLP-1-respons veel lager was na het korrelbrood vergeleken met controlebrood. Dus, zoals waargenomen in alle studies, vertoonde de RaE een sterke correlatie met GIP, maar was voor GLP-1 deze relatie minder duidelijk. Ondanks vergelijkbare glucosekinetiek na controlebrood en korrelbrood, werd de GLP-1-respons duidelijk beïnvloed door de aanwezigheid van gebroken tarwekorrels. Mogelijk speelt stimulatie van GLP-1 door galzuren hierbij een rol.

Samengevat onthullen deze bevindingen de complexe metabole processen die postprandiale glycemie en insulinemie bepalen na de consumptie van zetmeelrijke voedingsproducten. Er werd waargenomen dat de glycemische respons in het algemeen geen '*in vivo* zetmeelverteerbaarheid' weergeeft na consumptie van vezelrijke tarweproducten bij gezonde mannen. Langzamere intestinale opname van glucose uit een zetmeelrijk voedingsproduct resulteerde in lagere postprandiale GIP- en insulineconcentraties, maar niet noodzakelijk in een lagere glycemische respons, vanwege een langzamere GCR. Dit toont aan dat de snelheid van '*in vivo* zetmeelvertering', die kan worden beïnvloed door factoren zoals de compactheid en/of de viscositeit van het voedingsproduct, beter wordt weergegeven door de GIP- en insulinerespons dan door postprandiale glycemie bij gezonde personen. Zelfs zonder de postprandiale glycemie te verlagen, kunnen deze producten gunstige langetermijneffecten hebben als gevolg van de gematigde postprandiale respons van bijvoorbeeld GIP en insuline. Langzame influx van glucose zou ook kunnen leiden tot minder oxidatieve stress en ontsteking, waardoor IR en het risico op het ontwikkelen van T2DM wordt verminderd. De GLP-1-respons werd bovendien onafhankelijk van de glucosefluxen beïnvloed, mogelijk als gevolg van andere kenmerken van het zetmeelrijke voedingsproduct, en kan verband houden met galzouten. Dit verdient verder onderzoek, aangezien GLP-1 en galzouten worden gezien als veelbelovende

therapeutische middelen bij T2DM en de preventie ervan.

Op basis van de bevindingen in dit proefschrift stellen we dat een meer compacte broodstructuur resulteert in een gematigde postprandiale respons, wat de gezondheid ten goede kan komen. Het benadrukt het belang van de ontwikkeling van zetmeelrijke voedselproducten met een langzamere afgifte en/of opname van glucose afkomstig uit zetmeel. Dit biedt mogelijkheden voor bakkerijen om hun werkwijzen voor het bakken van brood te verbeteren om de gezondheid te bevorderen, en zou bijvoorbeeld de meer traditionele manieren van brood maken kunnen stimuleren.

Concluderend zou de kwaliteit van zetmeelrijke voedselproducten en hun impact op de gezondheid niet alleen gebaseerd moeten zijn op de glycemische respons, aangezien veel andere postprandiale variabelen tegelijkertijd veranderen, en onderliggende responsen kunnen verschillen. We stellen daarom voor om gelijktijdig insuline en incretinehormonen te meten. Veranderingen in broodstructuur en -ingrediënten kunnen het postprandiale metabolisme en de gezondheidsgerelateerde uitkomsten beïnvloeden. Daarmee zijn het belangrijke factoren waarmee rekening moet worden gehouden bij de preventie van het ontstaan van metabole ziekten zoals T2DM.

Addendum

Dankwoord

DANKWOORD

Ja, het is zover! Dat het ooit af zou komen heb ik eigenlijk nooit betwijfeld, maar wanneer…!? Het was een grote uitdaging, vooral die 'laatste loodjes', maar ik ben erg blij dat het afgerond is en dat dit het laatste onderdeel is dat voor dit proefschrift geschreven wordt. En ik ben vast niet de enige die blij is dat het klaar is! Sommigen begonnen er maar niet meer over, anderen vroegen er voorzichtig of juist heel direct naar, een enkeling hoefde ik maar aan te kijken…Vele bemoedigende woorden en succeswensen heb ik mogen ontvangen, af en toe een schop onder mijn kont. Graag wil ik hier alle mensen bedanken voor hun bijdrage aan het tot stand komen van dit proefschrift in welke vorm dan ook!

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~

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Lieve Hilco, liefde is….(wat zou ik hier ook alweer invullen ☺). Heerlijk hoe we altijd lekker gek kunnen doen, waardoor ik thuis het werk toch snel (een beetje) vergeet. Bedankt voor de ruimte die je me hebt gegeven en de steun die je hebt geboden om deze klus te klaren. Helaas moesten onze vakanties en weekenden er vaak voor wijken, en werd ik er geen leuker mens van. Mooi om te zien dat je je passie hebt gevonden en dat je er vol voor gaat. Nu zijn de rollen omgedraaid en ben jij 24/7 aan het werk op de meest onmogelijke tijden; dat moet wel een mooie film worden! Lief dat je even tijd maakte om te helpen met de voorkant. Hopelijk vinden we binnenkort toch even tijd om weer samen op reis te gaan en de bioloog uit te hangen (dieren spotten!).

Coby

Addendum

About the author

ABOUT THE AUTHOR

Coby Eelderink was born on November 12th 1983 in Doetinchem, and grew up in Zelhem, the Netherlands. After graduating from secondary school (Ulenhof College, Doetinchem) in 2002, she moved to Groningen to start her studies in Biomedical Sciences at the University of Groningen. During her master phase she completed two Master variants: 'Research' and 'Business & Policy'. Her two research internships were on pregnancy-related topics. The first project, on identifying an immunosuppressive protein in ovarian follicular fluid, was performed at the department of Medical Biology, UMCG, in the group of Prof. dr. Paul de Vos under supervision of Dr. Marijke Faas. The second project, about the expression of Thromboxane receptors in normal and diseased placenta, was performed at the Kolling Institute of Medical Research in Sydney, Australia, in the Perinatal research group of Prof. dr. Jonathan Morris under supervision of Dr. Anthony Ashton. The business-related internship was about screening methods for probiotic bacteria and gut health, performed at TNO Quality of Life in Zeist, and supervised by Dr. Alwine Kardinaal (TNO), Dr. Koen Venema (TNO) and Prof. dr. Anton Scheurink (RUG). This project increased her interest in 'nutrition and health'. She graduated 'Cum Laude' in 2009 and started her PhD project at the Center for Medical Biomics, UMCG, under supervision of Prof. dr. Roel Vonk and Dr. Marion Priebe. She was involved in several nutritional intervention studies with human volunteers studying carbohydrate metabolism. The studies described in this thesis were part of the Top Institute Food and Nutrition (TIFN)-project entitled 'Slow starch' (B-1003). For one of the papers she won the TIFN Publication Prize 2012. During her PhD-project, Coby presented the results of her work at several national and international conferences. In 2012 she was awarded the Foppe ten Hoor Price for her presentation at the Dutch Nutritional Science days.

Since 2014 she is appointed as a 'postdoctoral' researcher at the department of Internal Medicine, division of Nephrology (UMCG), in the group of Prof. dr. Stephan Bakker, mainly conducting clinical trials in the field of nutrition and health.

A

Addendum

Abbreviations

LIST OF ABBREVIATIONS

A	Arabinose
APE	Atom percent excess
AX	Arabinoxylan
BA	Bile acid
CA	Cholic acid
CB	Control bread
CCK	Cholecystokinin
CDCA	Chenodeoxycholic acid
CI	Confidence interval
CV	Coefficient of variation
CVD	Cardiovascular disease
dAUC	Decremental area under the curve
DCA	Deoxycholic acid
EGP	Endogenous glucose production
FB	Flat bread
IFG	Impaired fasting glucose
FXR	Farnesoid X receptor
GC/C/IRMS	Gas chromatography combustion isotope ratio mass spectrometry
GC/MS	Gas chromatography–mass spectrometry
GCR	Glucose clearance rate
GH	Glycoside hydrolase
GI	Glycemic index
GIP	Glucose-dependent insulinotropic polypeptide
GL	Glycemic load
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
G_{RA}	Rapidly available glucose
G_{SA}	Slowly available glucose
G_{T}	Total glucose
G_{TA}	Total available glucose
iAUC	Incremental area under the curve
IGT	Impaired glucose tolerance
IR	Insulin resistance
IS	Insulin sensitivity
KB	Kernel bread
LCA	Lithocholic acid
LC/MS	Liquid chromatography mass spectrometry
LOQ	Limit of quantitation
OGTT	Oral glucose tolerance test
PA	Pasta

PBB	Bread with purple wheat bran
PLS	Partial least squares
QC	Quality-control
RaE	Rate of appearance of exogenous glucose
RaT	Rate of appearance of total glucose
RDS	Rapidly digestible starch
RS	Resistant starch
ROS	Reactive oxygen species
RR	Relative risk
RSD	Relative SD
SCFA	Short chain fatty acid
SD	standard deviation
SDS	Slowly digestible starch
SEM	Standard error of the mean
SGLT-1	Sodium glucose linked transporter
T2DM	Type 2 diabetes mellitus
TCA	Tauro cholic acid
TGR5	G-protein coupled bile acid receptor (also known as GPBAR1)
UDCA	Ursodeoxycholic acid
VAS	Visual analogue scale
WU	Water-unextractable
XRT	X-ray microtomography
X	Xylose